# "EVALUATION OF ANALGESIC, ANTIPYRETIC AND ANTI-INFLAMMATORY EFFECTS OF ETHANOLIC LEAVES EXTRACT OF *Luffa acutangula (L.) Roxb.*"

***A Dissertation Submitted To***

**THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI.**

***In partial fulfilment of the requirement for the award of the degree of***

**MASTER OF PHARMACY**

**(PHARMACOLOGY)**

***Submitted by***

**Reg. No. 261525401**

***Under the Guidance of***

**Mrs. V. LALITHA, M. Pharm.,**

**Assistant Professor,**

**Department of Pharmacology**

**OCTOBER-2017**

**NANDHA COLLEGE OF PHARMACY AND RESEARCH INSTITUTE, ERODE – 638 052, TAMILNADU**

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# NANDHA COLLEGE OF PHARMACY

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**Dr.T.Sivakumar, M.Pharm., Ph.D.,**
**Principal**


Date 11.09.2017

## CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"EVALUATION OF ANALGESIC, ANTIPYRETIC AND ANTI-INFLAMMATORY EFFECT OF ETHANOLIC LEAVES EXTRACT OF *Luffa acutangula* (L.) Roxb"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, was carried out by **Mr. ADITH PILLAI** in the Department of Pharmacology, Nandha College of Pharmacy, Erode-52 for the partial fulfillment for the degree of **MASTER OF PHARMACY** in Pharmacology under the supervision of **Mrs. V.LALITHA, M.Pharm.,** Assistant Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode.

The work is original and has not been previously formed the basis for the award of any other Degree, Diploma, Associateship, Fellowship or any other similar title and the dissertation represent entirely an independent work on the part of the candidate.


**Mrs. V.Lalitha, M.Pharm.,**
Assistant Professor,
Department of Phamacology,
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Erode- 52. (T.N)

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This work is original and has not been submitted in part or fulls for other degree or diploma any university.

**Place:** Erode **Mrs. V. Lalitha, M.Pharm,**

**Date:**

# EVALUATION CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"EVALUATION OF ANALGESIC, ANTIPYRETIC AND ANTI-INFLAMMATORY EFFECTS OF ETHANOLIC LEAVES EXTRACT OF *Luffa acutangula (L) Roxb.*"** Submitted to "THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI, was carried out by **ADITH PILLAI,** in the department of Pharmacology, Nandha College of Pharmacy, under the supervision and guidance of **Mrs. V. LALITHA, M.Pharm., Assistant Professor**, Department of pharmacology, Nandha College of Pharmacy, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university.

**Internal Examiner** **External Examiner**

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**Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)**
**Institutional Animal Ethics Committee (IAEC)**
Reg No: 688 / 02 / C – CPCSEA

## CERTIFICATE

| | | |
|---|---|---|
| Title of the project | : | Evaluation of analgesic, antipyretic and anti-inflammatory effects of ethanolic leaves extract of *Luffa acutangula* (L.) Roxb. |
| Proposal Number | : | NCP/IAEC/No: 2016-17/02 |
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| Expiry date (Termination of the Project) | : | 02- 04- 2017 |
| Name of the IAEC / CPCSEA Nominee | : | Dr.K.Balasubramanian |

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सं. भा.व.स./द.क्षे.के./No. BSI/SRC/5/23/2017/Tech. /691 दिनांक/Date: 7th July 2017

सेवा में / To

Mr. Adith Pillai
Second Year M. Pharm.
Department of Pharmacology
Nandha College of Pharmacy
Erode – 638 052

महेदय/Sir,

The plant specimen brought by you for authentication is identified as *Luffa acutangula* (L.) Roxb. (= *Cucumis acutangulus* L.) - CUCURBITACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

(डॉ सी मुरुगन/Dr. C. Murugan)
वैज्ञानिक 'डी' एव कार्यालय अध्यक्ष /
**Scientist 'D' & Head of Office**

वैज्ञानिक 'डी' / SCIENTIST 'D'
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# DECLARATION

The work presented in this thesis entitled **"EVALUATION OF ANALGESIC, ANTIPYRETIC AND ANTI-INFLAMMATORY EFFECTS OF ETHANOLIC LEAVES EXTRACT OF *Luffa acutangula (L) Roxb*"** was carried out by me in the Department of Pharmacology, Nandha College of Pharmacy, Erode-52 under the supervision and guidance of **Mrs. V. LALITHA, M.Pharm.,** Assistant Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university.

**Reg.No.261525401**

**Place: Erode**

**Date:**

Final Year M.Pharm.

Department of Pharmacology

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## ACKNOWLEDGEMENT

"***Success is the progressive realisation of worthy goal many able people failed to achieve anything worldwide because they have not been properly guided. Success of any project depends solely on support, guidance and encouragement received from the guide and well wishers".***

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# CONTENTS

# INTRODUCTION

## 1. INTRODUCTION.

Plants are one of the important sources of medicine. The application of plants as medicine dates back to prehistoric period. Several indigenous drugs used in modern medicine have figured in ancient manuscripts such as Rigved, the Bible, and Quran. Over six thousand years ago, the ancient Chinese were the first to use the natural vegetation as medicine. In India, the Ayurvedic system of medicine has been in use for over three thousand years. Hippocrates, the 'Father of Medicine' was the first to give a scientific explanation of diseases. Indian system's of medicine includes Ayurveda, Siddha, Unani, Tibetan and Naturopathy. Herbal therapy provides rational means for the treatment of many internal diseases which are considered to be obstinate and incurable in other systems of medicine. It aims at both the prevention and cure of diseases.

## 1.1 HISTORY OF HERBAL DRUGS

Thousand years ago many natural drugs were identified for combating human ailments either by instinct or intuition or trial and error methods. The earliest mention of medicinal use of plants has been used found in '*Rig Veda'*, which was written between 4000 and 1600 B.C. In the "*Atharva Veda",* we find the more varied use of drugs. It is the "*Ayurveda* "which is considered as an "Upa *Veda "* that definite properties of drugs and their uses have been given in great detail. "*Charka samhita"* is another earliest treatise on "Ayurveda" (600 BC), which lists a total of 341 plant produce for use health management. *"Sushruta Samhita"* also dealt with plants related to medicine. *Dhanvantari* and *Nagarjuna* were the well known person with an intimate knowledge of the characteristics of medicinal plants. *Rauwolfia,* which has acquired world wide popularity, finds mention in ancient Hindu scriptures as well as in the monumental work of *charaka.* The plant was mentioned as usefull antidote for snake bites and insect stings. (1)

Their effectiveness, easy availability, low cost, and comparatively being devoid of serious toxic effect (time tested) popularized herbal remedies. Medicinal plants have their values due to the presence of chemical constituents, commonly known as secondary metabolites,

present in various plant tissues. These substances are alkaloids, glycosides, essential and fatty oils, resins, gums, mucilage, tannins etc. of large use. These active principles may be present in the organs of the plant, viz-roots, seeds, leaves, bark, wood etc... [2]

Popularity of herbal medicine over allopathic medicine is because of the following reasons:-

- Low cost of medicine
- Relatively free from side effects
- Cures the root cause and remove it
- Cures for many obstinate diseases
- Easy availability [3]

## 1.2 SCOPE OF HERBAL MEDICINES:

Several civilizations across the globe have herbs in different forms. Before the advent of modern medicine herbs were used in the form of powders, pastes, tablets, galenicals like tinctures, infusion, decoction, oils saturated with herbs, oils, poultice etc. Herbs have been used in India in Ayurveda, Sidda, Unani systems of medicine, collectively known as indigenous system of medicine. The modernized method of formulating herbal medicines has yielded a new system of herbal medicine, popularly known as phytomedicine. The Ayurvedic medicines can be classified as like Charak samhita, Sushrut samhita, Bharat Bheshaj Ratnakar etc. and Ayurvedic proprietary medicine.

In developed countries, particularly in the United States of America, the herbal formulations are included in the list of food supplements and are considered as alternative or complementary system of medicines. Several clinical trials on herbal extract have been undertaken to prove their safety and efficacy using protocols acceptable to the modern medicines. Many developed countries have realized the usefulness of herbs as a sourse of starting material (lead compounds) in the process of drug discovery. The chances of getting new drugs from herbs are more than getting anew drugs from synthetic source because of availability of literature on plant activity. Many biomarkers have been isolated and identified

There is fundamental difference between the indigenous system of medicine and modern medicine. The modern medicine makes use of single ingredient whereas the indigenous system of medicine believes that different constituents of herbs have synergistic effect. It has been shown that mode of action differs when effect of isolation biomarkers is compared with the total extracts. [4]

## 2. PAIN

Algesia (pain) is an ill-defined, unpleasant sensory and emotional experience associated with actual or potential tissue damage, which varies from person to person and in the same person, from time to time. Unrelieved acute pain can cause chronic pain and long standing pain can cause anatomical and even genetic changes in the nervous system. Pain is warning signal, primarily protective in nature, but causes discomfort and suffering. Excessive pain may produce other effects such as sinking sensation, apprehension, sweating, nausea, palpitation, and rise or fall in BP, tachypnoea

### 2.1 TYPES OF PAIN

- Stimulation of a nociceptor, due to a chemical, thermal, or mechanical event that has the potential to damage body tissue, may cause nociceptive pain [5]
- Damage to the nervous system itself, due to disease or trauma, may cause neuropathic (or neurogenic) pain. Neuropathic pain may refer to peripheral neuropathic pain, which is caused by damage to nerves, or to central neuropathic pain, which is caused by damage to the brain, brainstem, or spinal cord.
- Nociceptive pain and neuropathic pain are the two main kinds of pain when the primary mechanism of production is considered. Nociceptive pain may be classified further in three types that have distinct organic origins and qualities.

1. **Superficial somatic pain (or cutaneous pain):** Is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminates just below the skin, and due to the high concentration of nerve

endings, produce a sharp, well-defined, localized pain of short duration. Examples of injuries that produce cutaneous pain include minor wounds, and minor (first degree) burns.

2. **Deep somatic pain:** It originates from ligaments, tendons, bones, blood vessels, fasciae, and muscles. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, aching, poorly-localized pain of longer duration than cutaneous pain: examples include sprains, broken bones, and myofascial pain.
3. **Visceral pain:** Originates from body's viscera, or organs. Visceral nociceptors are located within body organs and internal cavities. The even greater scarcity of nociceptors in these areas produces pain that is usually more aching or cramping and of a longer duration than somatic pain. Visceral pain may be well localized, but often it is extremely difficult to localize, and several injuries to visceral tissues exhibit "referred" pain, where the sensation is localized to an area completely unrelated to the site of injury.[6]

## 2.2 CAUSES OF PAIN

Pain can be caused by many different factors. Conditions that accompany normal aging may affect bones and joints in way that cause pain. Normal body pain is formed due to some common health conditions such as,

- Muscle Knots
- Sensitization
- Brain pain
- Pathological sensitization
- Vitamin D deficiency
- Muscle tension and contracture
- Referred pain
- The pain of stuckness
- Drug side effect, especially bisphosphonates and statins
- Analgesic rebound.[7]

## 2.3 DIAGNOSIS OF PAIN

The medical community is full of tests to try to finds the cause of pain or disease. However, the real cause of your chronic, severe pain may be difficult to diagnose using labs and diagnostic tests. Imaging tests are also called radiological test. With these tests, the doctor uses different technologies to get a better picture of what's going on in the body-with the bones, soft tissues, and organs.

Here are the most common imaging tests:

- **X-ray**
- **CT scan**
- **MRI**
- **Nerve blocks**
- **Discography**
- **Myelogram**
- **Ultrasound imaging** [8]

## 2.4 TREATMENT OF PAIN

Pain is complex, so there are many treatment options - medication, therapies, and mind –body techniques. Some measures you can take to relives pain from injuries

- Resting the area of the body where you are experiencing aches and pains.
- Applying ice to the affected area to help relives pain and reduce inflammation.
- Taking an over-the- counter pain reliever, or **analgesic drugs** such as Acetaminophen, NSAIDs such as aspirin, ibuprofen etc. [9]

## 2.5 ANALGESICS

A drug that selectively relives pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. A wide range of drugs are used to control pain. They range from mild over-the-counter (OTC) drugs, such as aspirin and acetaminophen, to

strong general anaesthetics. Drugs that relieve pain often reduce fever and inflammation that are used to treat conditions such as

- ❖ Mild to moderate pain caused by injury or surgery
- ❖ Fever, headaches, and painful menstruation
- ❖ Rheumatoid arthritis ( a chronic inflammatory disease of the peripheral joints)
- ❖ Osteoarthritis (a chronic disease that involves wear and deterioration of joints in the body, causing inflammation)
- ❖ Chronic pain associated with cancer, AIDS, multiple sclerosis, or sickle cell disease

Some commonly used **Analgesic drugs** are

- **Acetaminophen**
- **Acetanilide**
- **Morphine**
- **Aspirin**
- **Caffeine**
- **Codeine**
- **Ibuprofen**
- **Tylenol**
- **Medical cannabis** [10]

## 2.6 CLASSIFICATION OF ANALGESICS

A drug that selectively relieves pain by acting CNS or on the peripheral pain mechanism. Without significantly altering consciousness is called an analgesic.

Analgesics are divided into two groups:

- Opioids /Narcotics/Morphine like analgesics
- Nonopioid/Non-narcotic/Aspirin like/antipyretic/anti-inflammatory analgesics

## 2.6.1 OPIOIDS ANALGESICS

It was introduced in Britain at the end of 17th century, usually taken orally as "Tincture of laudanum". These are the substance that can be endogenous or synthetic, which produce morphine like effect. It is a dark brown, resinous material obtained from poppy (papaver somniferum), capsule.

It contains two types of alkaloids:

A. Phenathrene derivatives

Morphine (10% in opium)

Codeine (0.5% in opium)

Thebaine (0.2% in opium)

B. Benzylisoquinoline derivatives

Papaverine (1% in opium)

Noscapine (6% in opium)

USES- Used for thousands of years to produce:

- Euphoria
- Analgesia
- Sedation
- Relief from diarrhea
- Cough suppression [11]

## 2.6.2 OPIOIDS RECEPTORS

There are 4 types of opioid receptors, with multiple receptor subtypes:

**Mu-** These receptors produce the most profound analgesia, and can cause euphoria,

**Kappa-** These contribute to analgesia at spinal level. These receptors trigger a lesser analgesic response,

**Delta-** These receptors modulate mu receptors activity

**Sigma-** These receptors provide little to no analgesia [11]

### 2.6.3 MECHANISM OF ACTION

All opioid receptors are G –protein coupled receptors, stimulation of these receptors inhibits adenylate cyclase resulting in decreased intercellular cAMP formation. They also facilitate the opening K+ channels leads to hyperpolarisation and inhibit the entry of calcium into the cell. In addition to this they inhibit the opening of calcium channels. All these results in a decrease in the intercellular calcium which, in turn, decrease the release of neuron transmission of pain impulses.

Opioids also directly inhibit the transmission in the dorsal horn ascending pathway. [12]

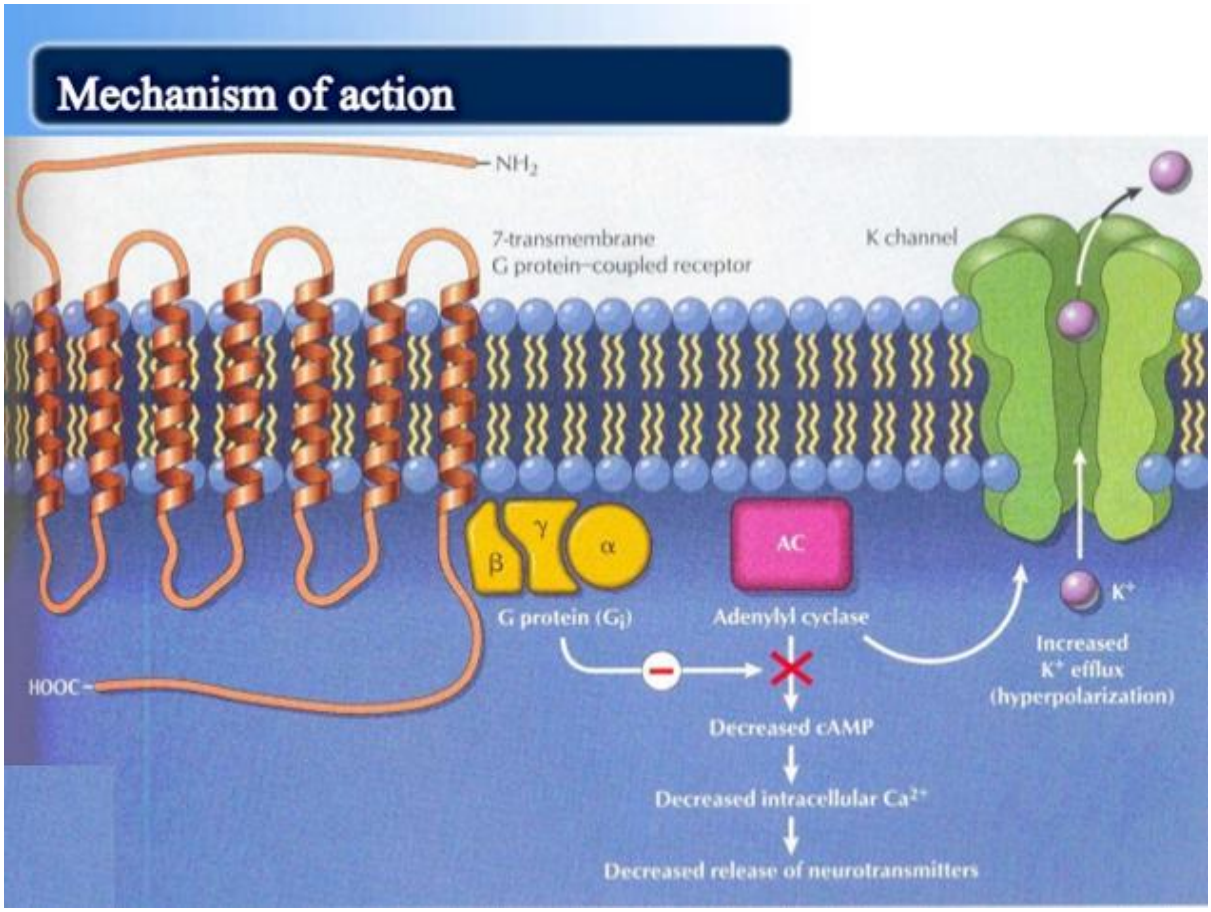


**Figure 1: mechanism of action of analgesic drug**

## 2.7 ANIMAL MODELS FOR EVALUATING CHRONIC PAIN

Animal tests are in the search for new analgesics are desinged as models for the treatment of pathological pain in man; but they usually differ from the orginal in that the drug is given before the noxious stimulus (thermal, electrical, chemical, and mechanical types of stimuli).[13] Hence, these tests only measure the power of a drug to increase the minimal stimulus requiredto elicit pain or nociceptive response. The methodology to perform these tests using above stimuli is described below.

- ❖ Hot plate methods
- ❖ The tail immersion test
- ❖ Writhing test

## 3. FEVER

Fever also known as **pyrexia** is defined as a temperature higher than 38.3ºC(100.9ºF) that lasts for more than three weeks with no obvious source despite appropriate investigation. It is a pathologic elevation of the normal body temperature; it is an active process and resists changes by the external enviornment. Fever is not a disease, but merely a sign of many different disease. Body temperature may be raised without pathological causes, as in excecise or in hyoerthermia resulting from excessive exposure to heat. Fever is usually a sign of infection in the body. Infections suchnasncolds and flu are very common, especially in preschool children. [14]

Fever is most often caused by virus and sometimes by bacteria. Viral infections are common and do not need antibiotics as they do not cure viruses. Bacterial infections are usually treated with antibiotics. It is the body's natural response to help fight infection. fever itself is not harmful, so it is usually not necessary to treat fever.

## 3.1 TYPES OF FEVER

There are mainly four types of fever based on the fluctuation of body temperature

- **Intermittent :** The body temperature alternates between fever and normal at regular intervels
- **Remittent :** The body temperature always above normal but fluctuates in wide ranges
- **Relapsing :** The fluctuations occurs between days. le : Week of fever followed by week of normal. [15]
- **Constant :** Minimal fluctuation. Always above normal

## 3.2 STAGES OF FEVER

1. **The chill phase:** or a period of raising temperature. A chill amy last few minites it may reach an hour.
   Patients experience a feeling of cold and shivering, skin is cool and pale
2. **The course of fever:** when the temperature is maintained at an elevated level. The skin feels warm and flushed
   The patients feels-thrist, malaise, weakness, aching muscle and drowing or restless.
3. **The terminatiohn orn decline period:** it is a periods when temperature falls to normal either by:

**a- Crisis:** the temperature falls quickly over a periods of few hours

**b- lysis:** the temperature falls gradually over a period of days or weeks

Ashort lysis takes about 3 days

A long lysis takesnfrom 7 to 10 days. [16]

## 3.3 CAUSE OF FEVER

- Exogenous pyrogens: derived from outside sn individual and may include LPS and toxins.
- Endogenous pyrogens: originating inside the body and many include pyrogenic cytokines, IL-1,IL-6,TNF.
- Damage to heat- regulating centre due to head injuries.cerebrovascular accidents and abnormally high body

temperature. This leads to disturbance in the heat regulating mechanisam.

- Acute infections disease.
- Acute and prolonged pain.
- Extreme nervousness.
- Emotional stress.
- Trauma or injury to body tissues. [17]

## 3.4 SIGNS AND SYMPTOM OF FEVER

- Rapid pulse
- Rapid shallow respiration
- Cold, then hot skin
- Headache
- Sweating and shaking chill
- Restlessness
- Anorexia
- Nausea and vomiting
- Dehydration
- Constipation
- Decrease urinary out put (oliguria)
- Sweating
- Appetite loss [18]

## 3.5 COMPLICATIONS OF FEVER

- Sinusitis
- Step throat
- Brochitis
- Pneumonia
- Brochiolitis
- Croup
- Sleep disruption
- Physical difficulities [19]

## 3.6 DIAGNOSIS OF FEVER

Q fever, caused by *Coxiella burnetii*, should be included in the differential diagnosis of acute neurologic disease in a patient with fever. Patients with acute Q fever infection have frequent neurologic symptoms, varying from a severe headache in most of the patients to confusion, but rarely meningitis.

The first step is accurate recording of core body temperature. A rectal thermometer is more reliable than skin surface or buccal recordings. The temperature is monitored constantly in an intensive care setting. Basic laboratory investigations include blood counts, serum electrolytes, blood urea nitrogen, creatine, and liver function tests. [20] Further investigations of the cause depend on the clinical features and suspected pathology. The predominant causes are infections. Gene expression profiles of blood leukocytes in febrile children can help to discriminate viral from bacterial causes of fever without an apparent source. Several biomarkers have been used to differentiate infections from inflammatory or malignant causes of fever, but commonly used biomarkers, such as C reactive protein, do not have enough sensitivity or specificity to guide treatment decisions, and procalcitonin is considered to be the most helpful laboratory biomarker for this purpose. [21] Cerebrospinal fluid is examined and cultured if meningitis is considered as a clinical diagnosis

## 3.7 TREATMENT OF FEVER

When the presence of fever is contra-indicated in a patient, what then are the most effective ways of reducing temperature? Traditional methods have relied on administering antipyretic drugs, such as paracetamol, and external cooling procedures, such as tepid sponging. When the skin is cooled by tepid sponging, the body detects a difference between the thermostatic set point and the peripheral thermoreceptors.[22] The warming response which this triggers to bring the temperature detected at the peripheral thermoreceptors to the thermostatic set point is counterproductive and can be distressing for the patient because of the violent shivering that can occur.

Antipyretics are drugs that decrease fever. Fever is a pathologic elevation of the normal body temperature; it is an active process and resists changes by the external environment. Fever is not a disease, but merely a sign of many different diseases. Body temperature may be raised without pathologic causes, as in exercise or in hyperthermia resulting from excessive exposure to heat.

In the SGH, the antipyretic drug of choice is paracetamol. Rosenthal and Silverstein (1988), say salycilates have been available and used to control temperature since the 1880s. Antipyretic drugs, such as aspirin, paracetamol and ibuprofen, do not stop the production or actions of cytokines but block the action of the enzyme cyclo-oxygenase which is essential for the production of prostaglandins in the brain. When the thermostatic set point has returned to normal, cooling begins and the body temperature falls. Antipyretic drugs also relieve arthritis and myalgia associated with fever by blocking the production of prostaglandins at the joints and muscles. In addition, they do not interfere with the immune response to pyrogen activity and are useful in relieving the discomfort of a feverish illness.

Some antipyretic drugs,

- NSAIDS Such as ibuprofen, ketoprofen and nimesulide
- Aspirin and related salicylates such as choline salicylate, magnesium salicylate and sodium salicylate
- Paracetamol ( USAN and JAN acetaminophen)
- Metamizole, banned in over 30 countries for causing agranulocytosis
- Nabumetone
- Phenazone (antipyrine), available in combination with benzocaine as an ear drop in the US

## 3.8 MECHANISM OF ACTION OF ANTI-PYRETIC DRUGS

Fever is a complex physiologic response triggered by infectious or aseptic stimuli. Elevations in body temperature occur when concentration of prostaglandin E (2) (PGE(2) increase within certain areas of the brain. These elevations alter the firing rate of neurons that control

thermoregulation in the hypothalamus. Drug that blocks pyrogen-induced prostaglandin production in thermoregulatory center (CNS). It will reduce the heat production. [23, 24]

## 4.INFLAMMATION

Inflammation or phlogosis is pathological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cell. Although it is defense can be induced, maintain or aggravate many disease. [25] It is a complex phenomenon, comprising of biochemical as well as immunological factors. Inflammation is recognized by following symptoms:

1. Rubor (redness)
2. Tumor (Swelling)
3. Calor (heat)
4. Dolor (pain)
5. Functio laesa (Loss of functions) [26]

## 4.1 TYPES OF INFLAMMATION

Depending up on thedefence capasity of he host and duration of response, Mainly three types of inflammation are recognized or inflammatory response occurs in two distinct phases, each apparently mediated by different mechanisms. Based on the course and duration, the inflammation can be called as:

### Acute:

Inflammation is the response of living to damage. The acute inflammatory response has 3 main functions.

- The affected area is occupied by a transient material called the acute inflammatory exudate. The exudate carries proteins, fluid and

cells from local blood vessels into th damaged area to mediate local defenses.

- ❖ If an infective causaitive agent (e.g. bacteria) is present in the damaged area it can be destroyed and eliminated by components of the exudate.
- ❖ The damaged tissue can bebroken down and partially liquefied, and the debris removed from then site of damage.

The cause of acute inflmmation may be due tophysical damage, chemical substances, micro-organisms or other agents. The inflammtory response consists of changes in blood flow, increased permeability of blood vessels and escape of the cell from into the tissues. It is characterized by local vasodilation and increased capillary permeability. It is immediate and early response to injuries agents have three major components.

1) Increase in blood flow.
2) Structural changes that leads to plasma protein and leukocytes into circulation.
3) Accumulation of leukocyte in focus of injury.

**Sub-Acute:**

The inflammation lasts for 1 to 6 weeks or more. The type that is neither acute nor chronic is termed as sub-acute inflammtion. It lasts longer as compared to acute inflammation. Microscopically vascular, exudative as well as proliferative changes of acute and chronic inflammation are present. Exudate chiefly consists of eosinophils, lymphocytes, plasma cells, histocytes and fibroblasts.

**Chronic**:

Chronic inflammation as consideredto be of prolonged duration (weeks or month) in which active inflammation, tissue destruction and attempts at healing are proceeding simultaneously.[27] Chronic inflammation frequently begins insidously as a low grade, smoldering after asymptomatioc response and examplesn include rhematoid arthiritis, atherosclerosis, tuberculosiis etc.

Chronic inflammation is characterized by:

a) Inflammation with mononuclear cells.
b) Tissue destruction
c) Repair by connective tissue replacements

## 4.2 MECHANISM OF INFLAMMATION:

Inflammation process is continues process operating through many mechanisms. Histopathological and biochemical studies of inflammation indicate that it involves two distinct events:

A. Vascular events
B. Cellular events (leucocytic infiltration)

It contains:-

a) Changes in vascular flow and caliber
b) Vascular leakage

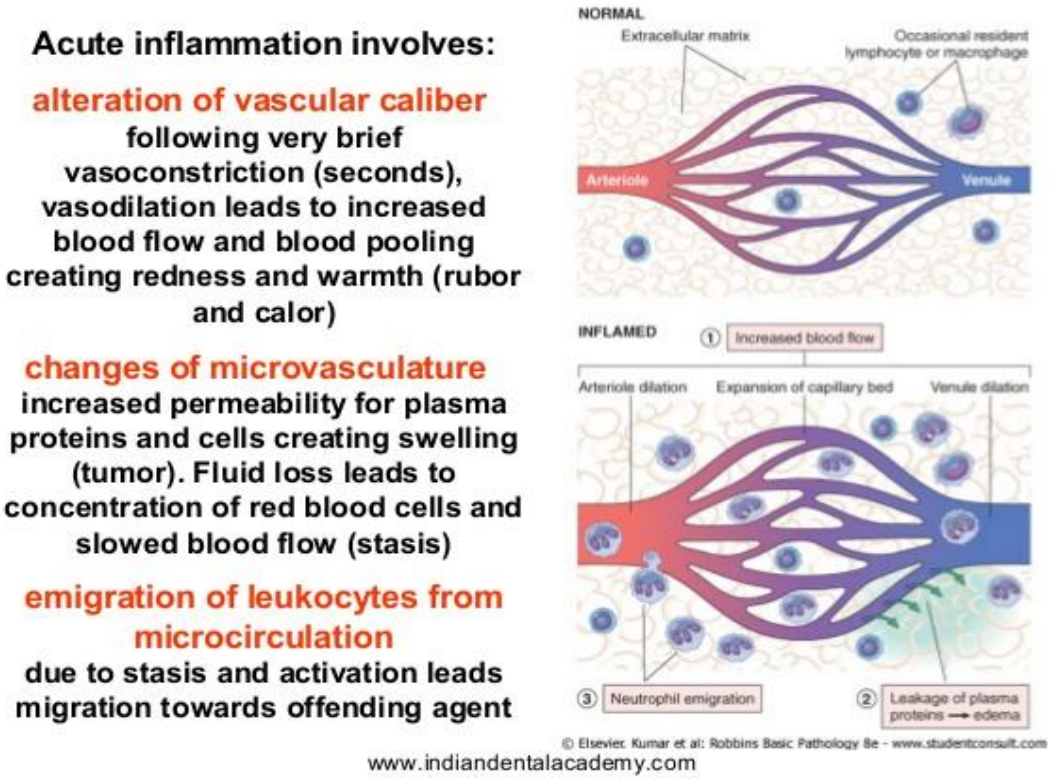


**Figure 2: process of inflammation**

**a) Changes in vascular flow and caliber:**

It begins early after injury and proceeds at different rates depending on severity of injury. Changes occurs in the following manner:

After an inconstsnt and transient vasoconstriction of arterioles, lasting few seconds, vasodilation occurs. Vasodilation first involves the arterioles and then results inthe opening of new capillary beds in the area. This along with increased blood flow, which is the casuse of heat and redness. Different mediator are involved in this process of vasodilation suchnas vasoactive amines, kinins and prostagladins.[28] Slowing of circulation is brought about by increased permeability of microvasculature, with outpouring of protein rich fluid into extravascular tissues.

- Stasis – develops due to loss of fluid, which results in coincentraion of red cells in small vessels and increased viscocity of the blood

- After this there occurs peripheral orientation of leukocytes,a process called leukocytic migration

**b) Vascular Leakage:**

Increased vascular permeability leading to the escape of protein rich fluid is the hallmark of acute inflammation. In normal conditions, fluid loss through the vascular bed is governed by the balance between hydrostatic and pressure in the venular end. Increase in capillary hydroststic pressure resulting from local vasodilation after an inflammatory injury drives fluid out of the vascular compartment and extra vascular spaces at the rate that is too rapid for reabsorption by lymphatic to prevent the resulting edema formation and tissue swelling.

Three phases of vascular permeability are [29]

I. Immidiate-Transient phase: This lasts about 30 min. Affects venules and is largely mediated by histamine.
II. Immediate- Prolonged phase: The exudation starts immediately but persists for days. It appears to be due to direct damage to vessels.

III. Delayed prolonged phase: Both capillaries and venules are affected by the direct injury of the agent(e.g heat) and by chemical mediators.

**B) Cellular Events:**

A critical function of inflammation is the delivery of leukocytes to the site of injury. Leukocytes ingest offending agents, kill bacteria and other microbes and degrade necrotic tissue & foreign antigens. Leukocytes may also prolong inflammation and induce tissue damage by releasing enymes, chemical mediators and toxic oxygen radicals. The migration of leukocytes at the sutes of inflammation takes place as follows

## 4.3 CAUSES OF INFLAMMATION

**Microbial infection:**

One of the most common causes of inflammation is microbial infection. Microbes include viruses, bacteria, protozoa, fungi, various parasites. Viruses lead to death of individual cells by intracellular multiplication, and either cause the cell to stop fuctioning and die, or cause explosion of the cell (cytolytic), in which case it also dies.

**Hypersensitivity reaction:**

A hypersensityvity reaction occurs when an altered state of immunolaogic responsiveness causes an inappropriate or expessive immune reaction that damages the tissues. The types of reaction will be disscussed in more detail later (in the lesson on immune mediated inflammation)

**Physical agents, irritant and corrosive chemicals:**

Tissue damage leading to inflammation may occur through physical trruma, ultravoilet or other ionizing radiation, burns or execssive cooling ('frostbite'). Corrosive chemicals(acid, alkalis, oxidizing,agents) provoke inflammtion through direct tissue damage. These chemical irritant cause tissue damage that leads directly to inflammation.[30]

**Tissue necrosis:**

Death of tissue from lack of oxygen or nutrients resulting from inadequate blood flow (infraction) is a potent inflammatory stimulus. The edge of recent infract often shows an acute inflammatory response.

## 4.4 DIAGNOSIS OF INFLAMMATION

Most commonly measured:

- Fever
- Cell Blood Count
- Erythrocyte Sedimentaion Rate
- C-reactive protein
- Procalcitonin [31]

## 4.5 TREATMENT OF INFLAMMATION

To reduce inflammation and the resulting swelling and pain, injured tissue need to be properly treated. The earilier you start traetment,the better.

The treatment of acute inflammation consist of **"R.I.C.E"** therapy- Which stands for Rest, Ice, Compression, Elevation. For acute inflammation in the foot or ankle, your foot ankle surgeon will recommend the following:

- **Rest:** Stay off of your foot as much as possible to prevent further injury. In some cases, complete immobilization may be required. Your doctor will decide whether you will need crutches and whether movement of your foot ankle is appropriate.
- **Ice:** Icing, which decreses blood flow to the tissue, thus reducing swelling and pain, should be continued until your symptoms resolve. Wrap ice cubes- or a bag of frozen peas or corn-in a thin towel and place the pack on the injured area for 20 mintues of each hour you're awake. If your skin turns blue or white, discontinue icing for a few hours. Two cautions: never apply ice or frozen bags directly to your skin. And never leave an ice pack on your injury while you sleep.

- **Compression:** keep the inflammed area compressed by wrapping it in an elastic bandage or stocking. Compression prevents additional fluid acumulation and helps reduce pain. Wrap the bandage more firmly at the toesand less firlmy at the calf. If your toes tingle or your foot throbs, the wraping may need to be loosening the wrap, contact your doctor as soon as possible.
- **Elevation:** keeping the foot elevated reduce the swelling by allowing excess fluid is to drain to the heart. The proper way to elevate your foot is to keep it level with or slightly above the heart. Place one or two pillows under your hip and knee are slightly bent. Never keep your leg extended straight out.[32]

  In addition to the above measures, your foot and ankle surgeon may prescribe a nonsteriodal anti inflammatory drug (NSAID), such as ibuprofen, or another type of medication.

Commonly used **anti inflammatory drugs**

- **Diclofenac**
- **Ibuprofen**
- **Indomethacine**
- **Ketoprofen**
- **Naproxen**
- **Sulindac**
- **Meloxicam**

## 4.6 MODEL USED FOR EVALUATING INFLAMMATIOn

Present day anti-inflammatory drug discovery is based on preliminary in vitro observations in a number of standard anti-inflammatory assays, in which the test compound produces unushally potent antagonism of inflammatory pathways such as the arachidonic acid pathways and some cytokine cascades. The effective candidate drug in invitro test is later tested in whole animal models of acute, subacute and chronic inflammation.[33]

- **Carrageenan induced paw edema model**
- **Cotton pellet induced granuloma**

# PLANT PROFILE

# PLANT PROFILE

## LUFFA ACUTANGULA

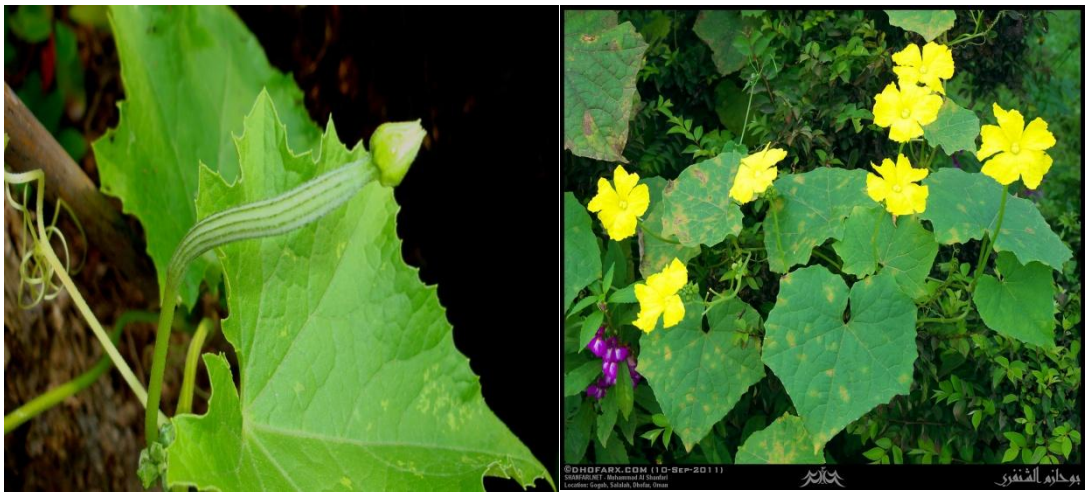

**Figure 3:** ***luffa acultagula*** **leaf**

**Biological Name : Luffa acutangula**

**Family : cucurbitaceae**

**Vernacular name**

**Hindi : kali.**

**Tamil : pirkankai.**

**Malayalam : pichenga.**

**Telugu : birakaya.**

**Plant Description**

**Colour : light green with brownish yellow.**

**Shape : somewhat twisted,wrinkled and angular, single layered epidermis, covered by thick cuticle; secondary cortex is wide in each ridge,**

**Size : petiole 3-8 cm long**

## Growth and Distribution

*Luffa acutagula (L).Roxb*. is a large monoeious annual climber. It is indigenous to western, central and southern regions of india, and regarded as wild variety of cultivated species. It has smaller leaves, flower, fruits and seeds. A large climber with palmately 5-7 angled or lobed leaves found wild in northwest india, bihar, bengal, sikkim and assam, and also in madras. seeds much compressed, 10-12 mm. Long, slightly corrugated on edges, black when rip. Mostly its culivated in a warm-season, cold – sensitive genus orginating in india. Propagation of *Luffa acutangula (L.) Roxb.* by seeds. *Luffa acutangula (L.) Roxb.* can grow in all type of soils and can be grown in rainy Season. Seeds can accordingly be down either in February- marth (or) june- July. Luffa is grown mostly as a novelty in florida grdens. However, some have been tried commertially for the sale of the sponges. Being cold sensitive, luffas should be grown during the warm season.

## Chemical constituents

The reported chemical examination of *Luffa acultangula (L.) Roxb*. showed the Presence carbohydrates, carotene, fat, protein, phytin, aminoacid, alanine, arginine, cystine, glutamicacid, glycine,hydroxyproline, leucine, serine, tryptophan,pipecolic acid. And also presence of alkaloids, carotenoidsm and terpenoid, flavonoids, tannins, luffangulin, sapogenin, oleanolic acid, cucurbetacin B,E and anthraquinones. Leaves are a healthy food and contains good amount of fiber,differents types of vitamins such as Vitamin B2, Vitamin C, Calcium, phosphorus,iron and small quantities of iodine and fluorine. Seeds shows presence of saturated and unsaturated fatty acid palmatic, stearic, oleic, linoleic and traces of lignoceric acid. Plant shows presence of oleanane type triterpene saponins- acutoside A, B, C, D, E, F, and G.

**Medicinal uses**

*Luffa acutangula (L.) Roxb.is* the source of many therapeutically important chemical constituents. Studies releaved its use in diabetes, immunomodulation, tumor suppresion, parkinsonism, antimocrobial, ulcer and hepatoprotection. And used as antioxidant, antipyretic, antiproliferative, anticataleptic, antimicrobial, analgesic and anti inflammatory agents.

# LITERATURE REVIEW

# REVIEW OF LITERATURE

A review of revealed literature is an essential aspects of scientific research. It involves the systemic identification, scrutiny and summaru of written material taht contains information on a research problem. It broadens the understanding and gain an insight of broad conceptual concept into which the problem fits.

**W. Kanwal et al., 2013[34]**

Ethanol extract of Luffa cylindrica (L.) Roem. Fruit peel was evaluated for antiemetic and anti-inflammatory effect using chick emesis model and carrageenan induced rat paw edema. The antiemetic effect was observed at a dose of 150 mg /kg body weight whereas anti-inflammatory effect was observed at doses of 500, 750 and 1000 mg /kg body weight orally. Chlorpromazine 150mg/kg and indomethacin 10mg/kg orally were used as standard anti-emetic and anti-inflammatory drugs. The anti-emetic effect was determined by calculating the mean decrease in number of retching in contrast with those of control after 10 minutes of copper sulfate (50mg/kg orally) administration. The degree of paw edema of all the groups was measured using a plethysmometer at 5th hour of carrageenan (1% w/v) administration. The extract exhibited statistically significant anti-emetic ($P< 0.001$) and anti-inflammatory ($P< 0.05$) effects.

**Jusal et al., 2008[35]**

The analgesic and hypoglycemic activities of *Bixa orellana*, *Kyllinga monocephala*, and *Luffa acutangula* were evaluated. Methanol extracts of *B. orellana* and *L. acutangula* showed significant hypoglycemic activities when administered 15 min after glucose load using a modified oral glucose tolerance test with Swiss Webster mice as test animals. An infusion of *B. orellana* was found to lower blood glucose level when administered 45 min before glucose load, while that of *K. monocephala* was found to lower blood glucose level when administered 15 min after glucose load. The methanol extract of *K. monocephala* was found to significantly reduce the number of writhes in mice administered

intraperitoneally with acetic acid to induce abdominal constriction, while those of *B. orellana* and *L. acutangula* did not.

**Nipun Dashora et al., 2013[36]**

Nature has been a source of medicinal agents for thousands of years, herbal medicines which formed the basis of health care throughout the world since the earliest days of mankind are still widely used. *Luffa acutangula* is widely growing vegetative climber and is used traditionally in folklore medicines for ailments including jaundice, diabetes, liver diseases, skin diseases, wounds etc. Taking into consideration, its medicinal importance and taxonomic confusion, exhaustive study of the morphology, tissue culture, phytochemical constituents, ethnobotany and biological activities of *Luffa acutangula var. amara* Roxb. fruits is carried out which will provide useful information in regard to its correct identity and help to differentiate from the closely related other species of *Luffa*.

**Ulaganathan Iyyamperumal et al., 2013[37]**

The aim of the present study was to evaluate the anti-inflammatory effect and *in vitro* antioxidant potential of ethyl acetate (EAELA) and ethanol (EELA) extracts of dried leaves of *Luffa acutangula (var) amara*. Anti inflammatory effect was evaluated by carrageenan induced hind paw edema and cotton pellet granuloma models. Anti oxidant effect was evaluated applying *in-vitro* models were the free radical scavenging activity was measured. Both extracts at both dose levels were found to possess significant anti-inflammatory effect in acute and chronic models. The EELA and EAELA extracts at 25 to 800 mcg/ml concentrations showed significant anti-oxidant effect in Nitric Oxide and DPPH models. Reactive oxygen species scavenging and lipid peroxidation inhibition activities indicate that *Luffa acutangula* might be valuable natural antioxidant source.

**Manikandaselvi et al., 2016[38]**

*Luffa acutangula* L. (Common name: Ridge gourd, Family: Cucurbitaceae) is a popular vegetable in India and other Asian countries. It is a healthy food and contains good amount of fiber, vitamins and minerals including Vitamin B2, Vitamin C, carotene, niacin, calcium, phosphorus, iron and small quantities of iodine and fluorine. *L. acutangula* has been used extensively in Indian traditional system of medicines as diuretic, expectorant, laxative, purgative, hypoglycemic agent and bitter tonic. Various biological activities of this plant were reported including its use in weight loss, jaundice, blood purification, hypoglycemia, and constipation, skin care, and immune system booster, wound healing, eye problems, stomach worms and asthma. The present review work focused on its distribution, botanical characters, ethnobotanical uses, folklore claims, nutritional value, phytochemical constituents, medicinal properties and biological properties of *L. acutangula*.

**Kalaskar Mohan G et al., 2010[39]**

The *Luffa acutangula* Linn. Var. *amara* Roxb. Is medicinal climber found in western, central and southern India, the fruits used in vata, kapha anemia, asthma, leucoderma, tumors, also useful as diuretic and in splenic enlargement. Scientifically it is proved as CNS depressant. The present study on pharmacognostical characters of *Luffa acutangula var. amara* Roxb. Fruits will be providing useful information in regard to its correct identity and help to differentiate from the closely related other species of *Luffa* and varieties *Luffa acutangula.*

**Dashora N et al., 2015[40]**

The objective of the present study is to explore the anticancer activity of the ethanolic and aqueous extracts of the *Luffa acutangula* in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line. Anticancer activity of ethanolic and aqueous extracts of *Luffa acutangula* was evaluated in EAC Swiss albino mice at the doses of 200 and 400 mg/kg body weight orally. Ethanolic and aqueous extracts showed

significant decrease in ($p<0.0001$) tumor volume, viable cell count, tumor weight and elevated the life span of EAC tumor bearing mice. Haematological profiles such as red blood cell (RBC), haemoglobin, and white blood cell (WBC) count reverted to normal level in treated mice. The results demonstrated that the extract has potent dose dependent anticancer activity comparable to that of cisplatin. Aqueous extract at both doses (200 and 400 mg/kg) and ethanolic extract at 400 mg/kg dose showed potent anticancer activity

**W. Kanwal et al., 2013[41]**

Ethanol extract of Luffa cylindrica (L.) Roem. Fruit peel was evaluated for antiemetic and anti-inflammatory effect using chick emesis model and carrageenan induced rat paw edema. The antiemetic effect was observed at a dose of 150 mg /kg body weight whereas anti-inflammatory effect was observed at doses of 500, 750 and 1000 mg /kg body weight orally. Chlorpromazine 150mg/kg and indomethacin 10mg/kg orally were used as standard anti-emetic anti-inflammatory drugs. The anti-emetic effect was determined by calculating the mean decrease in number ofretching in contrast with those of control after 10 minutes of copper sulfate (50mg/kg orally) administration. The degree of paw edema of all the groups was measured using a plethysmometer at 5th hour of carrageenan (1% w/v) administration. The extract exhibited statistically significant anti-emetic ($P< 0.001$) and anti-inflammatory ($P< 0.05$) effects.

**A. V. Misar et al.,2004[42]**

The fruits of Luffa acultangula Var. amara C.B. C Clarke were collected in winter season from western ghat area. These fruit were dried, powdered, defatted with ethanol. HPTLC pattern of ethanol extract was recorded after removal of solvent and was studied for effect on behavioural changes, exploratory activity, barbiturates sleeping time, using appropriate standards im mice. The extract exhibited dose-dependent CNS depressant activity.

**Sanjay Kumar et al., 2013[43]**

Neem(*azadiachta indica*), popularly know as 'wonder tree' is an evergreentropical tree, which has been reported to have beneficial effects on various disease pathology from the ancient time. Such. Among all ingredient azadirachtin, nimbidin, flavonoids, triterpinoids are not able for their beneficial effect aganist disease pathology. Neem has reported to have anti-allergenic, anti-dermatic, anti-feedent, anti-fungal, anti-inflammatory, anti-pyorrhoeic, anti-scabic mode of actions. Experimentalm observation releaved that NLE have analgesic, anti-inflammatory and antipyretic effect in albino rats.

**Sumanta Mondal et al., 2009[44]**

The methanol extract of *Neolamarckia cadamba* (family: Rubiaceae) barks significant analgesic, anti-infalmmatory and antipyretic activity. The acute toxicity, orally evaluated in mice, was found to be higher than 3000 mg/kg. The anti -inflammatory activity using carrageenan and antipyretic activity in yeast-induced pyrexia in rats. Were also examined. The extract at the dose 400 and 600 mg/kg significantly reduced the numbers of writhings at all tested doses (200,400 and 600 mg/kg p.o) The percentage inhibition of edema due to injection of carrageenan was found to be in promising result with 200 mg/kg dose level. Although these result provide a support for the traditional uses of N.cadamba barks, further studies are nessessary to better evaluate its safety and mode of action.

**Shahbaa et al., 2015[45]**

The aqueous extract of Parsley (Petroselinum *crispum*) were investigated for anti-inflammatory, analgesic and antipyretic activity at the doses of 2 , 5 and 10 g/kg of body weight. The experimental paradigms used were carrageenan, dextran, histamine induced pedal edema and cotton pellet induced granuloma for anti-inflammatory activity, while hot plate and acetic acid induced writhing methods were used to assess analgesic activity. Yeast-induced hyperpyrexia was used to evaluate the antipyretic activity. The extract also produced significant

($P < 0.01$) analgesic activity in both paradigms. In addition, the aqueous extract of parsley potentiated the morphine and aspirin induced analgesia. A significant ($P < 0.01$) reduction in hyperpyrexia in rat was also produced by the extract. This study exhibits that methanol extracts of leaves of parsley possess anti-inflammatory, analgesic and antipyretic activities.

**Saba et al., 2011[46]**

Ethanolic extract of the leaf of *Calotropis procera* was investigated for its anti-inflammatory and analgesic activities. The extract was evaluated using formalin-induced paw lick, carragenaan-induced paw oedema in Wistar rats, acetic acid-induced writhing and tail flick tests in mice. Each experiment consisted of thirty animals randomly, The administration of the extract was repeated for formalin-induced paw lick and acetic-acid induced writhing models in the presence of an opioid antagonist, naloxone. Inhibition of formation of paw oedema by the extract (100mg/kg b.w) was significantly higher than for Indomethacin. Itching was significantly reduced in rats administered with extract in the early phase of formalin response, and was comparable to Indomethacin (10mg/kg b.w). 100 mg/kg body weight of the extract also inhibited the writhing movement comparably with aspirin (15mg/kg b.w). Same pattern was also observed with tail flick model in mice

**Onasanwo et al., 2012[47]**

*Anacardium occidentalis* (family: Anacardiaceae) is a plant of the tropical climate widely used by folklore to treat pain and inflammation. This study was conducted to evaluate the analgesic and anti-inflammatory effects of the leaf extracts in rat and mice using different models. The extract prolonged the latencies of tail withdrawal to a similar degree as pentazocine. The extract caused significant inhibition of carrageenan induced paw oedema in rats ($P < 0.05$) in a dose dependent manner. Phytochemical analysis showed that the leaf extracts contain alkaloids, tannins, saponins and cardenolides.

**Safari et al., 2016[48]**

*Aloe volkensii* has been used to manage several diseases including pain, inflammation and fever. The aim of this study therefore is to investigate the analgesic, antipyretic and anti-inflammatory activities of its aqueous extracts. Analgesic activity was determined by use of 0.05 ml of 2.5% formalin−induced writhing test. A writhe was recorded by a stopwatch. Anti-inflammatory activity was established by a formalin induced inflammation test. Hourly changes in paw sizes and reduction of edema around the paw was determined using a vernier calipers. Antipyretic activity was carried out using 0.03 g of 10 ml/kg of 15% w/v Brewer's yeast-induced pyrexia. Temperature of each mouse was determined rectally by thermal probe thermometer. The results support the traditional use of *A.* volkensii in the treatment of various diseases associated with pain, fever and inflammation

**Neeraj Kumar Saini et al., 2012[49]**

To evaluate the analgesic, anti-inflammatory and antipyretic activity of methanolic Tecomaria capensis (T. capensis) leaves extract using different models in rats. Methanolic T. capensis leaves extract (100, 300, 1000 and 2000 mg/kg body weight) orally to observe acute toxicity, and observed for 14 days. Analgesic activity was evaluated using tail immersion and formalin induced paw licking models in rats. Anti-inflammatory activity was evaluated using carrageenan induced paw edema model in rat. Anti-pyretic activity was evaluated by using brewer's yeast induced by pyrexia model in rats. Results demonstrated that the no mortality was reported even after 14 days, this indicated that the methanol extract was safe up to a single dose of 2000 mg/kg body weight. This study

**Khadem Ali et al., 2012[50]**

To explore the efficacy of ethanolic leaf extract of Typhonium trilobatum L. Schott in treating diarrhea, pain and inflammation using experimental models. In the present study, acetic acid-induced writhing, xylene-induced ear edema and castor oil-induced diarrheal model were used to evaluate the analgesic, anti-inflammatory and anti-diarrheal activities, respectively. In anti-diarrheal test, the extract significantly

decreased the frequency of defecation and increased the mean latent period (P<0.01) in castor oil-induced diarrheal model mice at the doses of 250 and 500 mg/kg body weight. These results suggest that the extract possesses significant analgesic, anti-inflammatory and anti-diarrheal activities that support to the ethnopharmacological uses of this plant.

**Jude E Okokon et al., 2010[51]**

The ethanolic root extract of *C. zambesicus* (27-81mg/kg) was evaluated for anti-inflammatory, analgesic and antipyretic properties in mice. The extract (27-81mg/kg) demonstrated a weak anti-inflammatory activity. However, asignificant (P<0.01-0.001) analgesic and antipyretic activities were observed in all the experimental models tested. The extract may be exerting its effects through central mechanisms. This finding confirms its ethnomedical use in the treatment of malarial-associated symptoms.

**Nwafor Paul A et al., 2010[52]**

The effect of ethanolic extract of *Smilax krausiana* (Smilaceae) leaf was investigated for its analgesic and anti-inflammatory activities in mice. It also reduced in dose-related manner inflammation induced by fresh egg albumin, carrageenin and capsaicin. These inhibitions were statistically significant (p<0.01 – 0.001). Though the mechanism of action of the extract is not fully elucidated, it may in part involve suppression of capillary permeability through neurogenic and non-neurogenic pathways as well as its narcotic potential. The extract's inhibition of all the models investigated in a non-specific manner supports the folkloric use of the plant

**Bokanisereme et al., 2013[53]**

The objective of this study was to evaluate the *in-vivo* anti-inflammatory, anti - pyretic, analgesic activities and phytochemical analysis of ethanol cassava leaf extract (ECLE). The leaf extract was prepared with ethanol filter and rotary evaporated and stored in desiccator. A different concentration (100,250,500mg/kg) of the leaf

extract was evaluated for their anti-inflammary, analgesic and anti-pyretic effect using carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats respectively. The extract at concentration100, 250,500mg/kg was able to inhibit carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats. Terpernoids, tannins, flavonoids, carotenoids were found present in the ECLE.

**Safari VZ et al., 2016[(54)]**

*Acacia nilotica* has been used to manage several diseases including pain, inflammation and fever. However, its efficacy has not been scientifically validated. The aim of this study therefore is to investigate the antinociceptive, antipyretic and anti-inflammatory activities of its aqueous extracts. Antinociceptive activity was determined by use of formalin−induced writhing test. A writhe was recorded by a stopwatch. Anti-inflammatory activity was established by a formalin induced inflammation test. Hourly changes in paw sizes and reduction of edema around the paw was determined using a venier calipers. Antipyretic activity was carried out using Brewer's yeast induced pyrexia. Anti-inflammatory and antipyretic potential of aqueous leaf extracts of *A. nilotica* in albino mice and will serve as good bio-resource for generating readily available herbal formulations that are more effective in the treatment of pain, Inflammation and fever conditions which are cheaper than the conventional synthetic drugs and have no side effects.

**Sumanta Mondal et al., 2016[(55)]**

*Macrothelypteris torresiana* is a species of fern native to tropical and subtropical region and belonging to family Thelypteridaceae. The present study was conducted to evaluate antipyretic, analgesic and anti-inflammatory activities of ethanol extract from *Macrothelypteris torresiana* aerial parts (EEMTAP) at doses of 200 and 400 mg/kg body weight, *per os*. Analgesic activity was evaluated against both thermal and chemical induced stimuli, which were evidenced from tail immersion, formalin induced paw licking and acetic acid induced writhing test. Antipyretic activity was performed by using the yeast induced hyper-

pyrexia method. Carrageenan induced rat paw edema and the cotton pellet granuloma model were selected for evaluating anti-inflammatory activities. EEMTAP also significantly decreased the rectal temperature of the rats. An ethanolic extract of *Macrothelypteris torresiana* possesses analgesic, anti-inflammatory and antipyretic activity which may be mediated by the central and peripheral mechanisms.

**Alphonsine Ramde-Tiendrebeogo et al., 2015[56]**

This study has been carried out to evaluate the *in vivo* anti-inflammatory, analgesic and anti-pyretic effects of an aqueous decoction and ethanol extract (95%) of *Ficus sycomorus* leaves. The anti-inflammatory effect was evaluated by the carrageenan-induced mice paw oedema model. The non-morphine type analgesic effect was evaluated trough acetic acid-induced writhing test. The morphine type analgesic activity was tested by mouse tail–flick test. The anti-pyretic activity was tested by induced hyperthermia in mice with brewer's yeast. At the same dose, the analgesic effect of the ethanol extract on abdominal writhings induced by acetic acid, The antipyretic activity of the extracts at the same doses was comparable to those of the LAS (300 mg/ kg b.w.) and hydrocortisone (10 mg/kg b.w.) from the third hour.

**Bairagi Shripad et al., 2012[57]**

*Ficus Microcarpa* L. is a medicinal plant used for the treatment of various body pains in India traditionally. The methanol extract of its leaves was investigated for its analgesic and anti-inflammatory activities in animal models. The extract at 50, 100 and 200mg/kg body weight reduced significantly the formation of oedema induced by carrageenan and histamine. The extract had a good analgesic effect characterized by a reduction in the number of writhes when compared to the control. Similarly, the extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin. Acute toxicity test showed that the plant may be safe for the pharmacological uses. This study has provided some justification for the folkloric use of the plant in

several communities for conditions such as pain, skin allergies and inflammations.

**Omoniyi et al., 2017[58]**

Aqueous root extract of Dalbergia saxatilis, Hook, f., (Leguminosae) (DS) is reported useful for toothache, pains, and fever, but not scientifically proven. This study determined its effectiveness in pain, inflammation, and fever, applying scientific models. The analgesic activity was measured by acetic acid writhing, tail flick, tail immersion, tail clip, hot plate, and formalin pain tests; anti-inflammatory effects were determined via carrageenan and dextran rat paw oedema tests; antipyretic activity was measured by Escherichia coli lipopolysaccharide (ECL) and turpentine in rabbits, and D-amphetamine sulphate (D-AS) pyrexia test in rats.Unlike morphine, DS did not produce significant prolongation of the reaction times in the hot-plate, tail immersion, tail flick, and tail clip tests. As well as significant antipyretic actions involving cyclooxygenase, a2 adrenoceptor and interleukin-1 b1 due to any of glycosides, saponins or phenolic tannins.

**Godwin Christian Akuodor et al., 2016[59]**

To investigate the the analgesic, anti-inflammatory and antipyretic activities of the methanolic leaf extract of *Maerua crassifolia* in mice and rats. Acetic acid-induced writhing and tail immersion methods were used to assess analgesic activity, while xylene and carrageenan-induced paw oedema methods were used to evaluate the anti-inflammatory effect of the leaf extract. Yeast and amphetamine-induced pyrexia were used to investigate the antipyretic activity. The leaf extract (100, 200, and 400 mg/kg) showed a dose dependent and significant ($P < 0.05$) inhibition of pain in acetic acid-induced writhing and tail immersion tests. The phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, tannins, steroids, resins, saponins and cardiac glycosides. The oral median lethal dose of the leaf extract was estimated to be greater than 5000 mg/kg in rats. The findings confirmed its ethno medical use in the treatment of pains and feverish conditions.

**Sourabie1 T S et al., 2012[(60)]**

The present study deals with evaluation of the anti-inflammatory and analgesic properties of a lyophilized leaf extract of *Argemone mexicana* Linn. On laboratory animal. The anti-inflammatory study was done by using carrageenan-induced paw edema method. It was found that lyophilized extract can be effective in acute inflammatory disorders and in that case, it showed significant anti-inflammatory dose-dependent effect($p<0,001$) at the dose level of 250 mg/kg and 500 mg/kg.The plant extract was equally tested for its analgesic potential by using the hot plate test method and acetic acid Writhing method. By the hot plate method, the drug extract showed significant ($p<0,001$) increased latency periodthan the control group at oral dose of 250 and 500 mg/kg.

# OBJECTIVE AND PLAN OF WORK

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Non–steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for their wide range of activity. However its side effects are high. Natural products from medicinal plants have more pharmacological significance with improved efficacy and lesser side effects

*Luffa acultagula (L.)Roxb.* Is an Indian traditional medicine used for analgesic, anti-inflammatory activity. Hence the present work was aimed to explore the use of extract of *Luffa acultagula (L.) Roxb.* With proper validation.

## PLAN OF WORK

- Literature survey.
- Collection of plan material and its authentication.
- Preparation of extract and preliminary phytochemical evaluation.
- Approval of protocol by institutional animal ethical committe. constituted as per the direction of CPCSEA to conduct animal study.
- Finding the dosage range of the extract as per OECD guidelines.
- Pharmacological studies.

**1.** Analgesic activity.

- ✓ Hot plate method in mice.
- ✓ Acetic acid induced writhing response in mice.
- ✓ Tail immersion test.

**2.** Anti-Pyretic Activity.

**3.** Anti-inflammatory activity.

- ✓ Carrageenan –induced paw edema in rats.
- ✓ Cotton pettel induced granuloma method in rats.

# MATERIALS AND METHODS

# MATERIALS AND METHODS

## PLANT COLLECTION AND AUTHENTIFICATION

### Plant collection

*Luffa acutangula plant was* collected in and erodes in the month march 2017

### Authentification

The plant was identified by Dr. C. Murugn, Scientist 'D' and Head of office, Botanical survey of India, Tamil Nadu Agricultural University Campus, and Coimbatore bearing the reference number BSI/SRC/5/23/2017/Tech/691.

### Drying and Pulverization of the plant material

After collection and authentification the leaves were washed to remove the dust Particles and allowed to air dry in a shade for complete drying. Then the dried leaves without moisture were powdered in a mixer grinder.

### Preparation of the plant extract

The coarse powder was packed tightly in the soxhlet apparatus and extracted with ethanol for 72 hours with occasional shacking maintained at 60$^{o}$c throughtout the extraction process. The extract was concentrated to of its original volume by evaporation. The resulting ethanolic extract of the *Luffa acutangula (L.) Roxb.* was subjected to phytochemical study.

### Phytochemical analysis

The ethanolic extract of *Luffa acutangula (L.) Roxb.* were subjected to qualitative phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, and free aminoacids and triterpenoids.

### 1. Test for carbohydrate

Small quantity of extract was dissolved in 5ml of water and filtered.

**Molisch test**

The filtrate was treated with a few drops of α- napthol (20% in ethyl alcohol). Then 1 ml of concentrated $H_2SO_4$ was added along the sides of inclined test tube and observed for formation of violet coloured ring at the interface.[61]

**2. Test for glycosides and anthroquinones**

**Borntrager's test**

A small amount of ethanolic extract was hydrolysed with hydrochloric acid for few hours on water bath and the hydrosylate was extracted with benzene. The benzene layer was treated with dilute ammonia solution and observed for the formation of reddish pink colour.

**Legal test**

The extract was dissolved in pyridine and made alkaline with few drops of 10% NaOH and freshly prepared sodium nitroprusside was added and observed for formation of blue colour.

**3. Test for flavonoids**

**Ammonia test**

Filter paper strips were dipped in the dilute solution of the extract, ammoniated and observed for colour change from white to yellow.[62]

**4. Test for Tannins and Phenolic compounds**

The extract was dissolved in distilled water and dissolved into three portions. Sodium chloride (10%) was added to one portion, 1% gelatine to second portion and gelatine salt reagent to third portion. Precipitation with later or both gelatin salt reagents was indicative of the presence of tannins. Precipitation with salt solution indicates a false positive test. Positive tests were further confirmed by the addition of a few drops of dilute ferric chloride (1%$FeCl_3$) to the test extract which gave blue or green black coloration.

**5. Test for Proteins and Aminoacids**

Small amount of extract was dissolved in distilled water and filtered.

**Biuret's test**

To the ammoniated alkaline filtrate add 2-3 drops of 0.002% copper sulphate and observed for appearance of red or violet colour.

**Millon's test**

To 2 ml of filtrate 5-6 drops of millons reagent (1 g of mercury + 9 ml of fuming nitric acid solution) was added and observed for red precipitates.

**Ninhydrin test**

To the filtrate lead acetate solution was added to precipitate tannins and filtered. The filtrate was spotted on paper chromatogram and sprayed with ninhydrin reagent and heated at 110ºC for five minutes and observed for red or violet colour.

**Xanthoprotein test**

To the filtrate a few drops concentrated nitric acid was added by the side of test tube and observed for appearance of yellow colour.

**6. Test for sterols and triterpenes**

The extract was refluxed with alcoholic potassium hydroxide until the completion of saponification. Then the mixture was diluted with distilled water and extracted with diethyl ether. The ethereal extract was evaporated and the unsaponifiable matter was subjected to the following tests.

**Libermann- Buchard's test**

The ether soluble residue was dissolved in chloroform and a few drops of acetic anhydride was added followed by a few drops concentrated sulphuric acid form sides of the test tube and observed for the formation of blue to blue- red colour.

**Salkowski's reaction**

To the ether soluble residue 2 ml of concentrated sulphuric acid was added and observed for the formation of yellow ring at the junction which turns red after one minute.

**Animal approval**

The study was conducted after obtaining from committee for the purpose of control and supervision on animals (CPCSEA) and institutional animal ethics committee (IAEC), proposal number NCP/IAEC/NO:02/2016-17

**Animals**

Swiss albino mice weighing 20-25 gm wistar rats weighing 150-200 gm were used for this study. The animals were obtained from animal house, Nandha College pharmacy, Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºc and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow. All the experimental procedures are protocols used in this study were reviewed by Institutional Animal Ethics Committe (688/02/C-CPCSEA) of Nandha college of pharmacy, proposal number (NCP/IAEC/NO: 2016-2017) and were accordance with the guidelines of the IACE

**Acute Toxicity Studies**

The acute toxicity was performed according to OECD guidelines 423. The selected albino rats were used for toxicity studies. The animals were divided into five groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded doses like 5, 50, 100, 1000 & 2000 mg/ kg body weight. Immediately, after dosing, the animals were observed continuously for first four hours for behavioural changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any. One-tenth and one-fifth

of the maximum tolerated dose (200 and 400 mg/ kg, body weight) of ethanol leaf extract of *Luffa acutangula((L) Roxb.* selected to evaluate analgesic, anti-inflammatory and antipyretic activity studies in rats[49].

# PHARMACOLOGICAL STUDIES

## ANALGESIC ACTIVITY

## HOT PLATE METHOD IN MICE

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally medicated analgesic effects of ethanolic extract of *Luffa acutangula (L.) Roxb.*The central analgesic drug pentazocine was used for positive control group. In this experiment, four groups (n=6) of Swiss albino mice (20-25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group I- Normal Control received CMC (0.5%), and Group II- standard treated with pentazocine (3 mg/kg i.p), whereas group III and IV- animals were treated orally with ethanolic extract of *Luffa acutangula (L.) Roxb.* (200 and 400 mg/kg respectively).

**Group I : Normal control (CMC)**

**Group II : Standard (Pentazocine 3 mg/kg)**

**Group III : Test Drug I (Ethanolic leaf extract of *Luffa acutangula)***

**Group IV : Test Drug II (Ethanolic leaf extract of *Luffa acutangula)***

Each animal was then individually placed gently on Eddy's hot plate at 55ºC. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate were determined at 30, 60, 90 and 120 min after administration of the drugs or vehicle[43].

## TAIL IMMERSION TEST

This method assessment was used to evaluate the centrally medicated analgesic effects of ethanolic extract of *Luffa acutangula (L) Roxb*. The wistar rats were divided into four groups each consists of six animals. They were placed into individual restraining cages leaving the tail hanging out freely. The lower 5cm portion of the tail is marked and this part of the tail was immersed in a water bath containing water at a temperature of 55± 0.5 ºC. Withdrawing the tail from the hot water showed the analgesic effect. The reaction time was noted on a stop-watch. Each animal served as control. The average of the two values was the initial reaction time. Group –II served as standard and received pentazocine (3 mg/kg, i.p) The Group III and IV were treated orally with ethanolic extract of *Luffa acutangula (L.) Roxb.* (200 mg/kg and 400mg/kg) respectively,

**Group I : Normal Control**

**Group II : Pentazocine (3 mg/kg)**

**Group III : Test Drug I (Ethanolic extract leaf *Luffa acutnagula*)**

**Group IV : Test Drug II (Ethanolic extract leaf *Luffa acutangula)***

The reaction time of the groups were taken at 0, 30, 60, 90 and 120min. The cut off time of the immersion was 15seconds. The reaction time was measured.

## ACETIC ACID INDUCED WRITHING RESPONSE IN MICE

This method was used to preferentially evaluate possible peripheral analgesic effects of ethanolic extract of *Luffa acutangula (L.) Roxb.* Four groups of Swiss albino male mice (n=6) were fasted overnight prior to start the experiment with free access to water. The peripheral analgesic drug Diclofenac sodium (10 mg/kg) was used as a positive control. Group-I Normal Control received CMC (0.5%) Group-II was treated with Diclofenac Sodium (10mg/kg), whereas Group III and IV were treated orally with the ethanolic extract of *Luffa acutangula (L.) Roxb.* at a dose of 200 mg/kg and 400mg/kg respectively.

**Group I : Normal Control**

**Group II : Diclofenac Sodium (10mg/kg)**

**Group III : Test Drug I (Ethanolic extract leaf *Luffa acutangula*)**

**Group IV : Test Drug II (Ethanolic extract leaf *Luffa acutangula)***

After 30 min of treatment, the mice were injected intra peritoneally with 0.1 ml of 1% acetic acid solution to induce the characteristic writhings. The mice were then placed in an observation box and the numbers of writhing were counted in a 5min period. The response of the extract and Diclofenac sodium treated groups was compared with those of animals in the control group [(44)].

## ANTI-PYRETIC ACTIVITY

The estimation of anti-pyretic efficacy of ethanolic extract was carried out using brewer's yeast induced pyrexia method. Fever was induced by means of subcutaneously injecting 10.0 ml/kg of a 20% w/v suspension of brewer's yeast in normal saline. Only animals whose rectal increased by at least 1.0º C after subcutaneously injecting 10.0 ml/kg of a 20%w/v suspension of brewer's yeast in normal saline. Only animals whose rectal temperature increased by at least 1.0ºC after 18 h of this yeast injection were included for the study. The normal rectal temperature of each animal was measured by using a flexible tail thermostat probe coated with lubricant, and temperature was recorded using a digital telethermometer. The experimental animals were randomly divided into four groups containing six animals in each group. The control group (I) was orally administered 0.5ml saline while the standard group (II) was given 150 mg/kg paracetamol and groups III and IV were prescribed dose I and II of methanol extract of test drugs, respectively [(43)].

**Group I : Normal Control (CMC)**

**Group II : Paracetamol (150mg/kg)**

**Group III : Test Drug I (Ethanolic extract leaf *Luffa acultangula*)**

**Group IV : Test Drug II (Ethanolic extract leaf *Luffa acultangula)***

The rectal temperature was recorded at time intervals of 1, 2, 3and 4 h after drug administration. Animals are rehabilitated with standards anti-pyretic drugs.

# ANTI-INFLAMMATORY ACTIVITY

## CARRAGEENAN-INDUCED PAW EDEMA IN RATS

For this experiment, the rats (120-150g) were divided into four groups (n=6). The group I received 0.5% CMC (10ml/kg), while the Group II received Indomethacin (10mg/kg). The Group III and IV were treated orally with the ethanolic extract of *Luffa acutangula (L.) Roxb.* at a dose of 200 mg/kg and 400 mg/kg orally.

**Group I : Normal Control (CMC)**

**Group II : Indomethacin (10mg/kg)**

**Group III : Test Drug I (Ethanolic extract leaf *Luffa acutanula*)**

**Group IV : Test Drug II (Ethanolic extract leaf *Luffa acutangula)***

Acute inflammation was produced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the sub planter region of the right hind paw of the rats. The animals were pre treated with the drug 1hour before the administration of carrageenan [51].The paw thickness was measured at 1, 2, 3 and 4 h after carrageenan injection by using digital vernier callipers.

## COTTON PELLET INDUCED GRANULOMA METHOD IN RATS

Cotton pellets, weighing 5mg each were sterilized. Under ether anaesthesia, the pellets were introduced subcutaneously through a skin incision on the back of the animals. Starting from 30 min after the implantation of cotton pellet for all the rats, Group-I normal control received CMC (0.5%) orally. Group-II was treated with Dexamethasone (1 mg/kg), whereas Groups III and IV were treated orally with 200 mg/kg and 400 mg/kg of ethanol extract of *Luffa acultangula (L.) Roxb*. [(48)].

**Group I : Normal Control (CMC)**

**Group II : Dexamethasone (1mg/kg)**

**Group III : Test Drug I (Ethanolic extract leaf *Luffa acultangula*)**

**Group IV : Test Drug II (Ethanolic extract leaf *Luffa acultangula)***

The test drugs were administered daily for 7days. On the 8$^{th}$ day, the animals were sacrificed with diethyl ether. The granulomas were removed and the weighed.

# RESULTS

# RESULTS

## 1. Qualitative phytochemical Evaluation of *Luffa acultagula (L) Roxb.*

**Table 1**

| Parameters | value |
|---|---|
| **1. Alkaloid** | + |
| **2. Carbohydrates** | + |
| **3. Glycosides** | - |
| **4. Flavonoids** | ++ |
| **5. Tannins & Phenolic compounds** | + |
| **6. Proteins & Amino acids** | + |
| **7. Saponins** | + |
| **8. Sterols or Triterpenes** | + |

**++: high content, +: moderate, - : Negative,**

From the qualitative phytochemical analysis of ethanolic extract of *Luffa acultagula (L)Roxb*. It content alkaloids, Tannins, phenolic compound, sterols, saponins, protein and amino acids and high amount of flavonoids.

# 1. Analgesic activity

## Hot plate Method in Mice

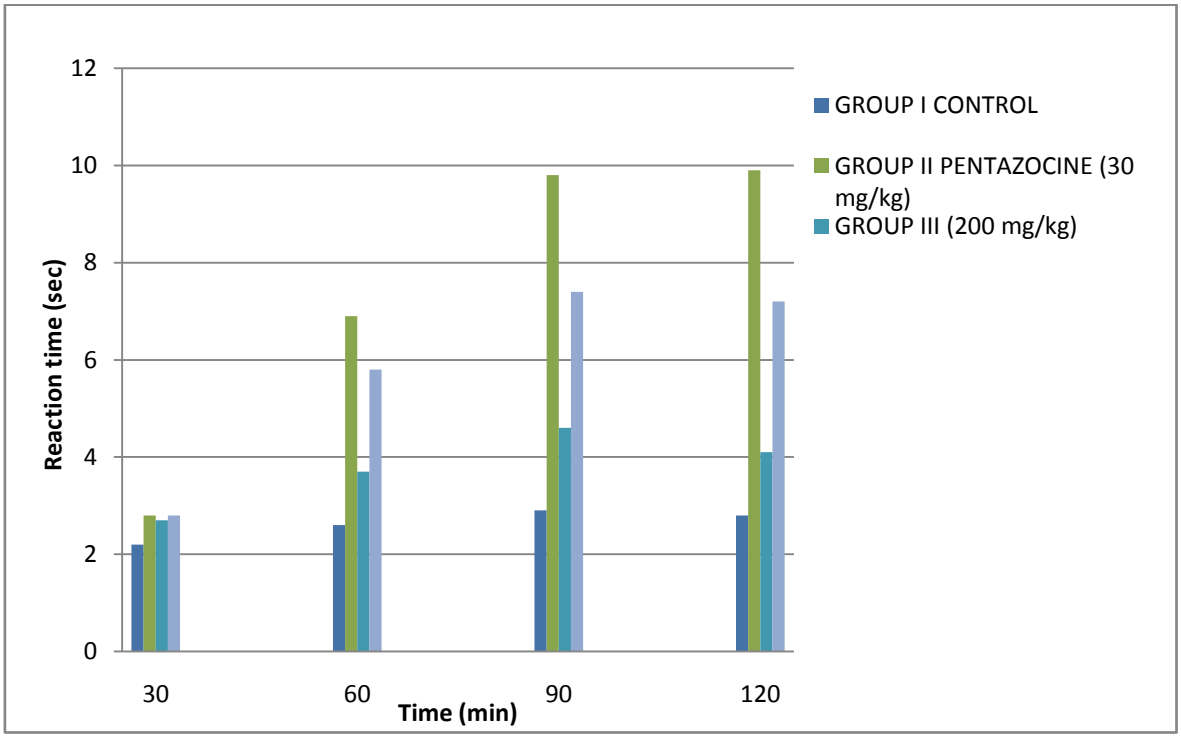
The analgesic activity of ethanolic leaves extract of *Luffa acutangula (L) Roxb.* was assessed using hot plate method in Swiss albino mice. The ethanolic leaves extract of *Luffa acutangula(L) Roxb*. Showed significant analgesic activity at 200 and 400 mg/kg. Analgesic activity was comparable with standard drug pentazocine. Among the two doses, 400 mg/kg showed maximum analgesic activity at reaction time 120 min (7.2±0.44) is slightly lower than the standard drug pentazocine (9.9±0.34) in this analgesic testing model, pentazocine significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally active drugs. In the present study, all extracts showed significant ($p<0.05$ and $p< 0.01$) analgesic activity but among the two doses, 400 mg/kg showed highest analgesic activity at reaction time120 min.

**Table: 2 Analgesic effect of ethanolic extract of of *Luffa acutangula (L) Roxb* on hot plate test in Swiss albino mice**

| GROUP | Paw licking or jumping in seconds | | | |
|---|---|---|---|---|
| | 30min | 60min | 90min | 120min |
| Group-I Control | 2.2±0.22 | 2.6±0.12 | 2.9±0.21 | 2.8±0.10 |
| Group-II Pentazocine(3 mg/kg) | 2.8±0.18 | 6.9±0.62** | 9.8±0.64** | 9.9±0.34** |
| Group-III ( 200mg/kg) | 2.7±0.20 | 3.7±0.15* | 4.6±0.21** | 4.1±0.41** |
| Group-IV (400mg/kg) | 2.8±0.14 | 5.8±0.37** | 7.4±0.39** | 7.2±0.44** |

Values were mean ± SEM, (n=6), *$P$<0.05 **$P$<0.01 Vs control.

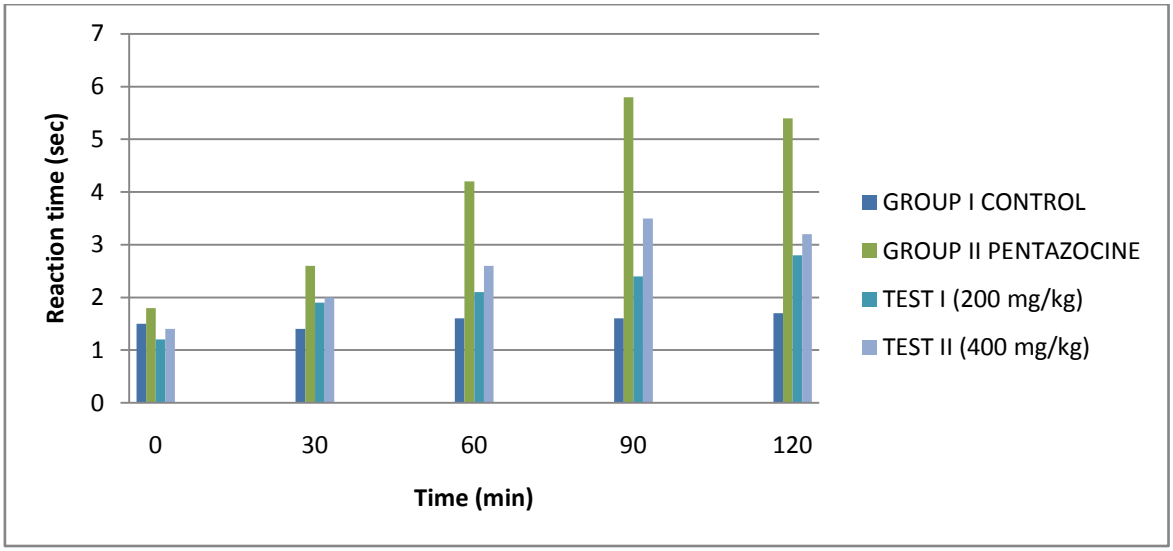
Data were analyzed by using One-way ANOVA followed by Dunnett's test.




Graph 1: Analgesic effect of ethanolic leaves extract of of *Luffa acutangula (L) Roxb* on hot plate method in mice.

## Tail Immersion Method

There was a significant reduction of pain full sensation due to tail immersion in warm water. The maximum inhibitory effect of *Luffa acutangula (L) Roxb*. Showed significant ($p < 0.01$) at 90 min post dose in 400 mg/kg. The maximum anti- nociceptive properties of the plant extract (3.5±0.04) were not as effective as that of pentazocine, 3 mg/kg (5.8±0.06)




Graphs 2: Analgesic Effect Of Ethanolic Leaves Extract Of *Luffa Acutangula (L) Roxb* On Tail Immersion Method In Rats.

**Table: 3 Analgesic effect of ethanolic leaves extract of *Luffa acutangula (L) Roxb on tail immersion method in rats***

| GROUP | Mean latency to tail immersion in seconds | | | | |
|---|---|---|---|---|---|
| | **0 min** | **30min** | **60min** | **90min** | **120min** |
| Group-I Control | 1.5±0.04 | 1.4±0.02 | 1.6±0.01 | 1.6±0.03 | 1.7±0.04 |
| Group II Pentazocine(3mg/kg) | 1.8±0.06 | 2.6±0.04** | 4.2±0.02** | 5.8±0.06** | 5.4±0.02** |
| Group III (200mg/kg) | 1.2±0.02 | 1.9±0.01* | 2.1±0.04* | 2.4±0.02 | 2.8±0.04* |
| Group IV (400mg/kg) | 1.4±0.01 | 2.0±0.04* | 2.6±0.01** | 3.5±0.04** | 3.2±0.01** |

Values were mean ± SEM,( n=6), *$P$<0.05 **$P$<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

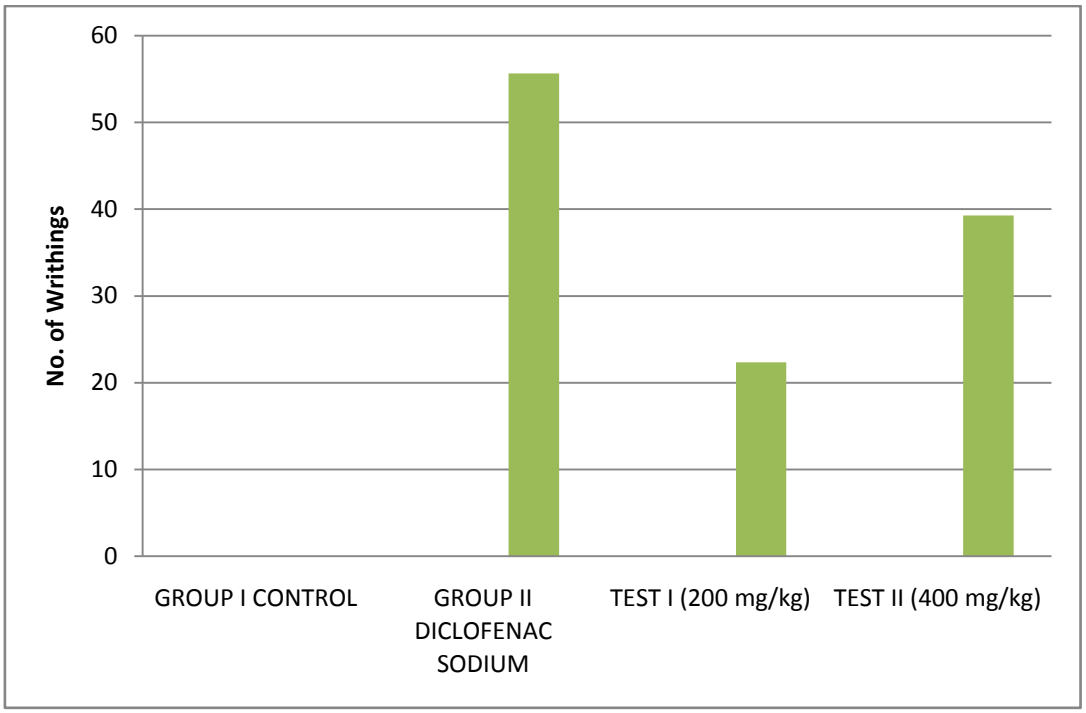
**Acetic Acid- Induced Writhing Response in Mice**

The oral administration of ethanolic leaves extract *o*f *Luffa acutangula (L) Roxb*. Showed a dose dependent analgesic activity. Injection of acetic acid into control mice produced 51.4±6.4 writhes. Pre-treatment with ethanolic extract of *Luffa acutangula (L.) Roxb*. at doses of 200 and 400 mg/kg reduced the number of writhes 39.4±2.4 (23.34 % protection) and 31.2±2.1 (39.29 % protection) respectively. Among the two doses 200, 400 mg/kg showed the slightly lower analgesic activity than standard drug Diclofenac Sodium 22.8±1.9 (55.64 % protection) it was observed that the onset of writhing was delayed and duration of writhing was shortened.

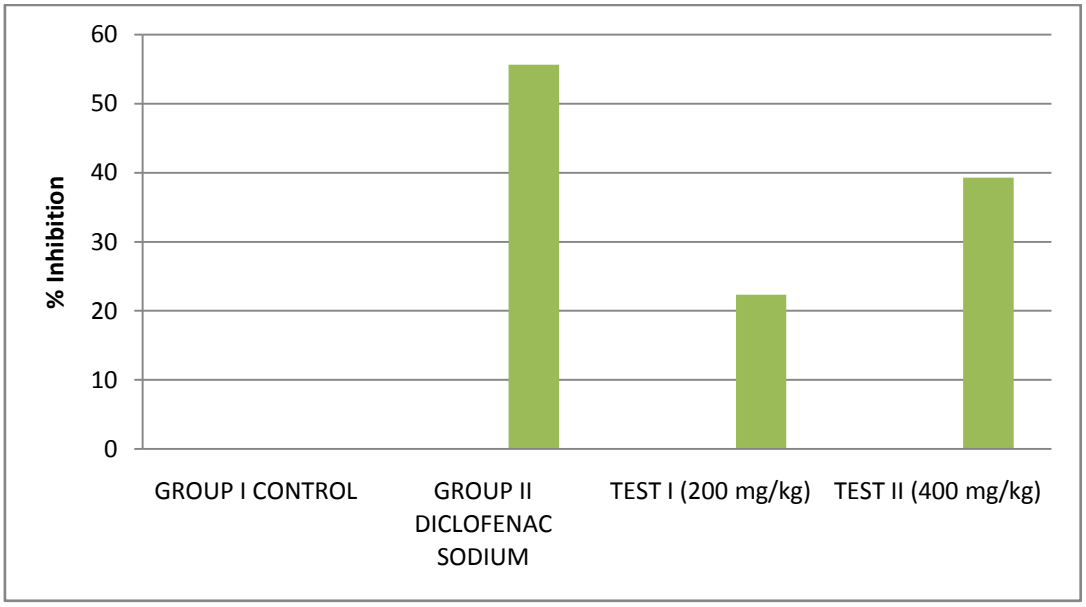
**Table: 4 Analgesic effects of ethanolic leaves extract of *Luffa acutangula (L) Roxb,* (on acetic acid writhing test in Swiss albino mice**

| GROUP | Number of writhes | % Inhibition |
|---|---|---|
| Group-I<br>Control | 51.4±6.4 | ___ |
| Group-II<br>Diclofenac Sodium<br>(10mg/kg) | 22.8±1.9** | 55.64 |
| Group-III<br>(200mg/kg) | 39.4±2.4** | 23.34 |
| Group-IV<br>(400mg/kg) | 31.2±2.1** | 39.29 |

Values were mean ± SEM, (n=6), ***P*<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.




Graph 3: Analgesic effect of ethanolic leaves extract of of *Luffa acutangula (L) Roxb*, on acetic acid induced writhing response in mice.




Graph 4: Analgesic effect of ethanolic leaves extract of of *Luffa acutangula (L) Roxb*, on acetic acid induced writhing response in mice. Results are expressed as a percentage of inhibition.

## 2. Anti-pyretic activity

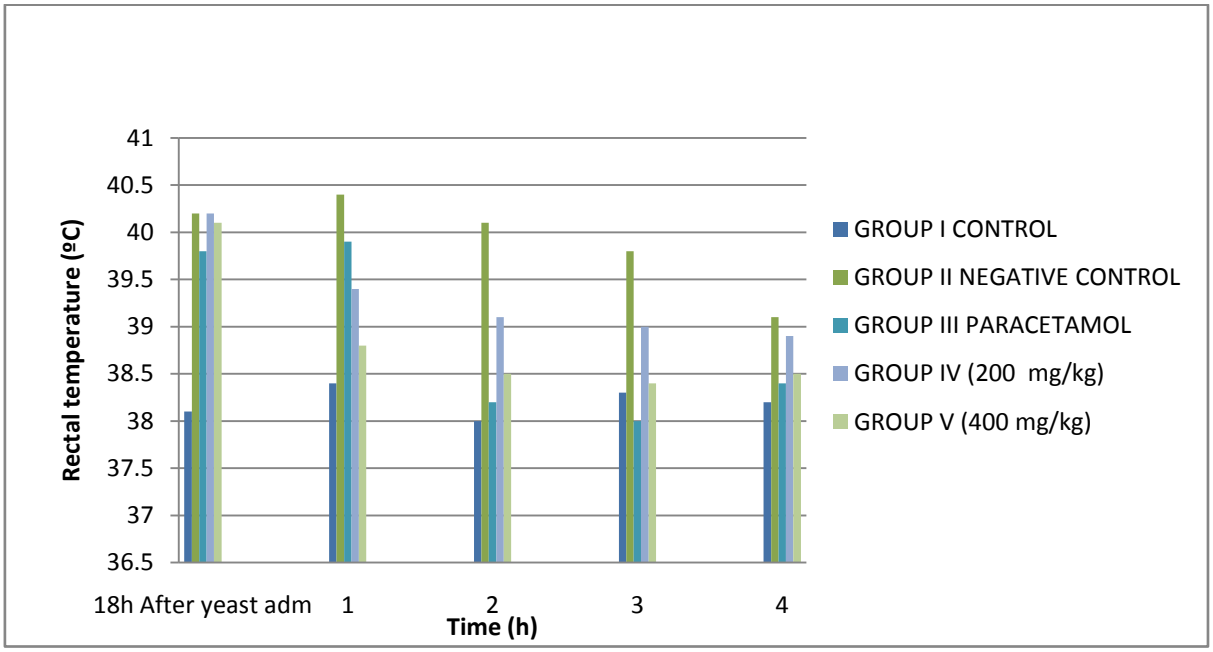
### Brewer's Yeast Induced Pyrexia in Rats

The anti-pyretic activity of ethanol leaves extract of *Luffa acutangula (L.) Roxb*. against yeast induced pyrexia is shown in Table 6. The ethanolic leaves extract of *Luffa cutangula (L.) Roxb*. at a doses of 200 and 400 mg/kg showed significant effect against Brewer's yeast induced pyrexia method. There was a progressive dose dependent reduction in the temperature of rats treated with the extract. The reduction caused by the extract was significant when compared to control.

**Table: 7 Anti-Pyretic Activity Of Ethanolic Extract Of *Luffa Acutangula (L) Roxb* On Brewer's Yeast Induced Pyrexia In Rats**

| Treatment | Rectal temperature (°C) | | | | |
|---|---|---|---|---|---|
| | 18 h after yeast administration | Temperature after treatment | | | |
| | | 1 h | 2 h | 3 h | 4 h |
| Group-I Control | 38.1±0.1 | 38.4±0.2 | 38.0±0.1 | 38.3±0.2 | 38.2±0.1 |
| Group-II Negative control | 40.2±0.1 | 40.4±0.3** | 40.1±0.2 | 39.8±0.1 | 39.1±0.3 |
| Group-III paracetamol | 39.8±0.2 | 37.9±0.2 | 38.2±0.2 | 38.0±0.1 | 38.4±0.2 |
| Group-IV (200mg/kg) | 40.2±0.1 | 39.4±0.3 | 39.1±0.1 | 39.0±0.2 | 38.9±0.3 |
| Group-V (400mg/kg) | 40.1±0.1 | 38.8±0.1 | 38.5±0.2 | 38.4±0.1 | 38.5±0.2 |

Values were mean ± SEM, (n=6), ***P*<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dennett's test.




Graph 9: Anti-Pyretic Activity of Ethanolic Leaves Extract of *Luffa Acutangula (L) Roxb*, On Brewer's Yeast Induced Pyrexia in Rats.

## 3. Anti-Inflammatory Activity

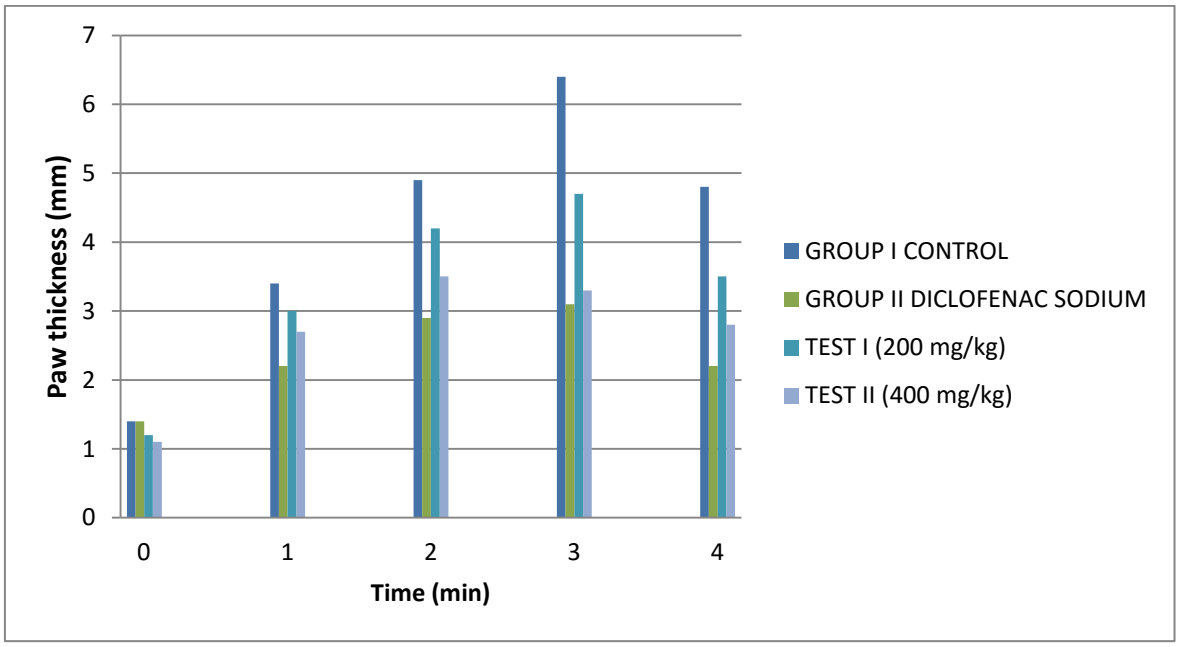
### Carrageenan-Inuced Paw Edema in Rats

The anti-inflammatory effect of the ethanolic leaves extract of *Luffa cautangula (L.) Roxb* on carrageenan – induced hind paw edema as shown in Table 4. The ethanolic leaves extract of Luffa *acutangula (L.) Roxb*. at doses 200 and 400 mg/kg produced a significant effect aganist carrageenan induced inflammatory effect . The dose of 400 mg/kg exhibited a significant inhibition of 48 % after 3 h, the effect increased after 3h (52%). Anti-inflammatory activity of ethanolic extract of *Luffa cautangula (L.) Roxb*. showed significant and similar to that of indomethacine (10 mg/kg).

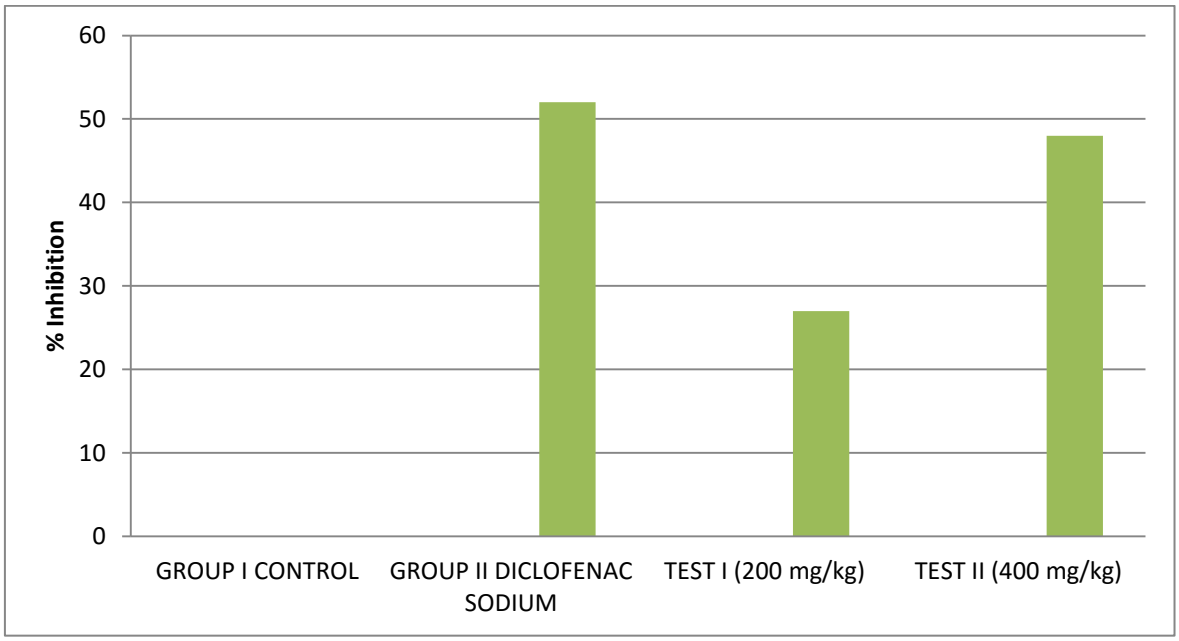
**Table: 5 Anti inflammatory activity of ethanolic extract of *Luffa acutangula (L) Roxb* on Carrageenan induced paw edema method in Wistar rays.**

| GROUP | Paw thickness in mm | | | | | % Inhibition at 3hr |
|---|---|---|---|---|---|---|
| | 0 hr | 1hr | 2hr | 3hr | 4hr | |
| Group-I Carrageenan (control) | 1.4±0.03 | 3.4±0.06 | 4.9±0.06 | 6.4±0.05 | 4.8±0.02 | ------ |
| Group-II Indomethacin (10mg/kg) | 1.4±0.04 | 2.2±0.03** | 2.9±0.04** | 3.1±0.02** | 2.2±0.04** | 52 |
| Group-III (200mg/kg) | 1.2±0.02 | 3.0±0.04 | 4.2±0.03 | 4.7±0.01* | 3.5±0.04** | 27 |
| Group-IV (400mg/kg) | 1.1±0.01 | 2.7±0.04** | 3.5±0.02* | 3.3±0.06** | 2.8±0.04** | 48 |

Values were mean ± SEM, (n=6), *$P$<0.05, **$P$<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test




Graph 5: Anti-inflammatory activity of ethanolic leaves extract of *Luffa acutangula (L) Roxb*, on carrageenan induced paw edema method in Wistar rats.




Graph 6: Anti-inflammatory activity of ethanolic leaves extract of *Luffa acutangula (L) Roxb*, on carrageenan induced paw edema method in Wistar rats. Results are expressed as a percentage inhibition.

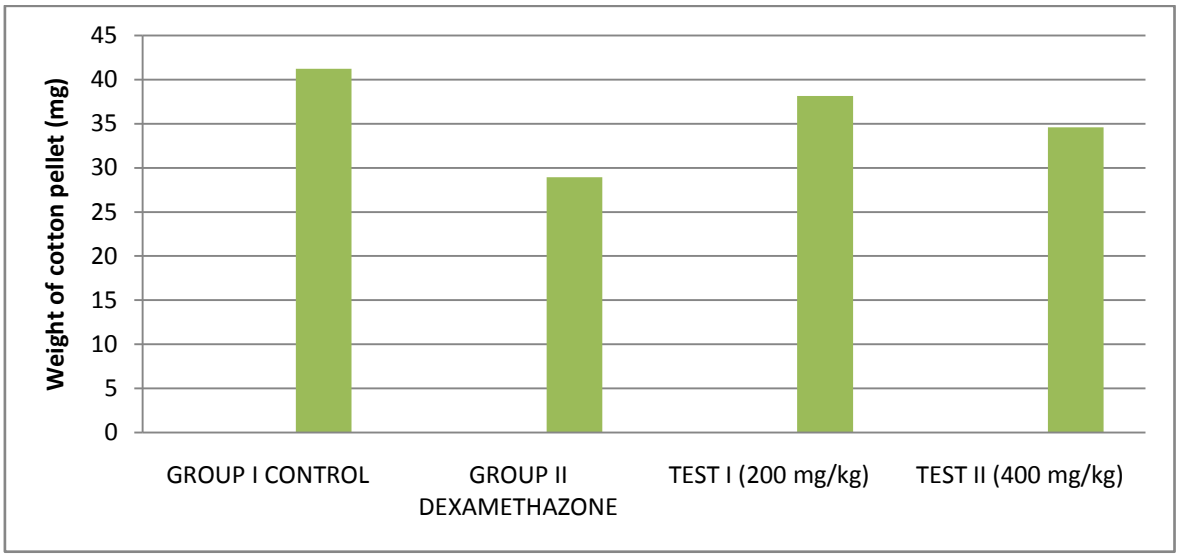
**Cotton Pellet-Induced Granuloma Method in Rats**

The anti-inflammatory effect of the ethanolic leaves extract *o*f *Luffa acutangula (L.) Roxb*. assessed by using cotton pellet induced granuloma method in Wistar rats. The ethanolic leaves extract of *Luffa acutangula (L.) Roxb.* Showed significant anti-inflammatory activity at 200 and 400 mg/kg dose. After 7 days, the mean weight of granulomatous tissue surrounding the threads was significantly lower for the group treated with *Luffa acutangula (L.) Roxb*. extract as compared to the control group. Among the two doses 400 mg/kg showed maximum decreased formation of granuloma tissue. The results indicate that Luffa acutangula (L.) roxb. at dose level of 200mg/kg and 400 mg/kg produced a significant decrease in the weight of granuloma 38.16±0.04 (7.4% inhibition) and 34.58±0.04 (16.1% inhibition) respectively. Among the two dose 400 mg/kg showed the slightly lower reduced weight of granumola than standard drug dexamethazone 28.92±0.04 (29.8% inhibition)

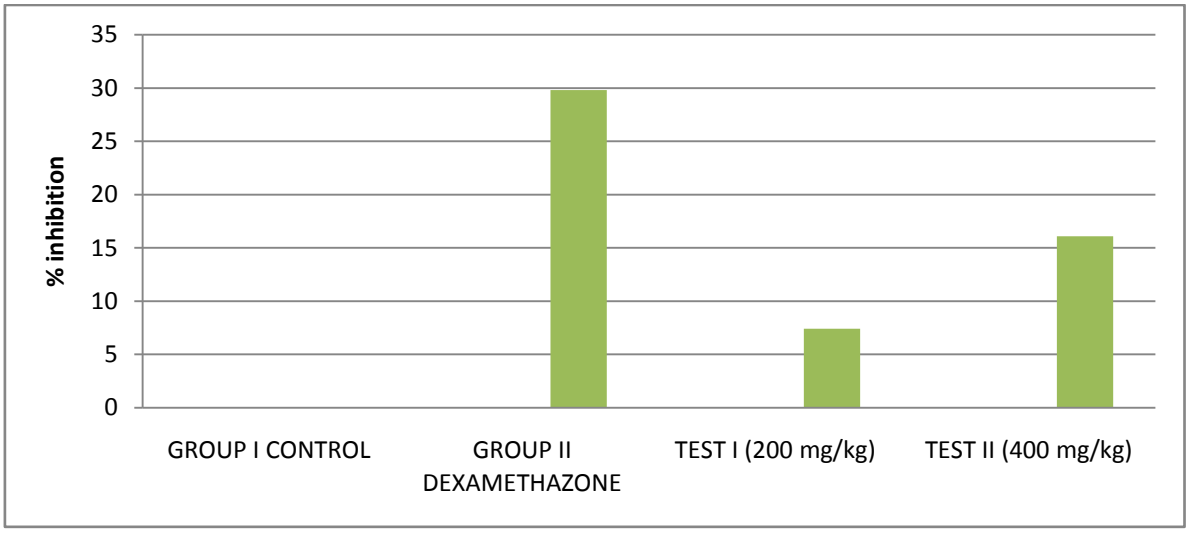
**Table.6 Anti inflammatory activity of ethanolic extract of *Luffa acutangula (L) Roxb*on cotton pellet induced granuloma pouch model Wistar rats**

| GROUP | Granuloma weight (mg) | % Inhibition |
|---|---|---|
| Group-I Control | 41.24±0.04 | ___ |
| Group-II Dexamethazone (1mg/kg) | 28.92±0.04** | 29.8 |
| Group-III 200mg/kg | 38.16±0.04** | 7.4 |
| Group-IV 400mg/kg | 34.58±0.04** | 16.1 |

Values were mean ± SEM, (n=6), ***P*<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dennett's test.




Graph 7: anti-inflammatory activity of ethanolic leaves extract of *Luffa acutangula (L) Roxb*, on cotton pellet-induced granuloma in rats.




Graph 8: anti-inflammatory activity of ethanolic leaves extract of *Luffa acutangula (L) Roxb*, on cotton pellet-induced granuloma in rats. Results are expressed as a percentage of inhibition.

# DISSCUSION

# DISCUSSION

The inflammation is complex process, which is frequently associated with pain and involves several events, such as the increase of muscular permeability, increase of granulocytes and mono nuclear cell migration, as well as the granulomatous tissue proliferation.[63] Pain is subjective experience, which is difficult to define exactly even though we all experience it. Pain distinguished as two types, peripheral or neurogenic pain may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by different inputs pain sensation.

The hot plate model was selected to investigate central antinociceptive activity because it has several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibits prostaglandin synthesis[64]. A number of phenolic compounds have been reported to produce analgesic activity. Other studies have demonstrated that various flavanoids such as rutin, quercetin, luteolin, biflavonoids and triterpenoids produced significant antinociceptive effect. As phytochemical test showed presence of flavonoids and tannins in ethanolic extract of *Luffa acutangula (L) Roxb*, they might suppress the formation of prostaglandin and bradykinins.

Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response[65]. The effect of the extract aganist the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animlas. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting anti-nociceptives. This response is thought to involve local peritoneal receptors. This result indicates that the analgesic effect of ethanolic extract of *Luffa acutangula (L) Roxb*, might be mediated by inhibiting the synthesis or action of prostaglandins.

The centrally acting analgesic activity of the extract was also corroborated in our study by tail immersion test results. The fact that in thermal stimuli (hot plate & Tail immersion tests), the anti nociceptive effect should be shown by acting centrally on opioid receptors. Since the drugs had shown the analgesic activity in tail immersion test, it seems that the ethanolic extract can act centrally. Taking this in to consideration the ethanolic extract of *Luffa acutangula (L) Roxb*, posses peripheral and central analgesic properties.

The ethanolic extract of *Luffa acutangula (L) Roxb* showed anti-inflammatory activity on an acute inflammatory process like in carrageenan induced paw edema in rats paw. It is well known that leukocytes migration to the injured tissues in an important aspect of the inflammatory process. Histamine and serotonin are responsible for the immediate inflammatory response, whereas kinins and prostaglandins mediate prolonged response. Anti-inflammatory activity of many plants has been attributed to their high sterol/triterpene or flavonoids content. The anti-inflammatory effect of ethanolic extract of *Luffa acutangula (L) Roxb.* in rats with carrageenan-induced paw was significant[(66)].

It is known that the inflammatory granuloma is a typical response of a chronic inflammatory process and it has been established that the weight of the pellets is well correlated with the granulomatous tissue. The chronic inflammation occurs by means of the development of prolifereative cells. These cells can be either spread or in granuloma form. The *Luffa acutangula (L) Roxb* extract showed significant anti-inflammtory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions. It reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide during granuloma tissue formation.

Brewer's yeast was used to induce fever in albino rats. Fever was recorded 18 hrs after yeast injection since yeast takes a total of about 18 hrs to cause the elevation of body temperature. Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect. Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins. The inhibition of prostaglandin synthesis

could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibitions of these mediators are responsible for the antipyretic effect.

The oral administration of *Luffa acutangula (L) Roxb* significantly attenuated rectal temperature of yeast induced albino rats. Thus it can be postulated that *Luffa acutangula (L) Roxb*, *contained* pharmacologically active principles that interfere with the release of prostaglandins. After three hours of the test period, the ethanolic leaves extract of *Luffa acutangula (L) Roxb* produced appreciable antipyretic activity against brewer's yeast induced pyrexia in albino rat. It was revealed that the extract showed dose dependent antipyretic activity.

# SUMMARY AND CONCLUSION

## SUMMARY AND CONCLUSION

The present thesis entitled "Evaluation of analgesic, antipyretic and anti-inflammatory effect ethanolic leaves extract of *Luffa acutangula (L.) Roxb."* deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Luffa acutangula (L.) Roxb*. belonging to the family Cucurbitaceae. The results obtained from the preliminary phytochemical screening of *Luffa acutangula (L.) Roxb.* extract showed the presence of flavonoids, alkaloids, tannins as shown in Table 1. It was reported that the flavonoids frequently found in plants posses analgesic, antipyretic and anti-inflammatory activity. The plant was collected and got authentification from Botanical Survey of India, southern regional centre, Coimbatore with the reference number BSI/SRC/5/23/2017/Tech/691. Approval was obtained from committee for the purpose of control and supervision of experimental animals (CPCSEA) and institutional animal ethics committee (IAEC), proposal number NCP/IAEC/NO: 02/2016-17.

The plant was shade dried and crushed. It was pulverized and extracted with ethanol using soxlet apparatus. The resulting extract was concentrated. The study of the plant *Luffa acutangula (L.) Roxb*. was done by using mice with the oral doses of 5, 50, 100, 1000 & 2000 mg/ kg body weight of extract and no mortality was observed for 24 hours. Thus dose was identified as per OECD 423 Guidlines.

As for the analgesic effect, the leaf extract appear to act via the central and peripheral mechanisms of analgesia by using hot plate, tail immersion and Acetic acid induced writhing animal model. Antipyretic activity of leaves extract was done by using yeast induced pyrexia method and finally anti-inflammatory effect of plant extract was done by using carrageenan- induced paw edema in rats and cotton pellet granuloma techniques.

The *Luffa acutangula (L.) Roxb.* has shown a significant anti-inflammatory, anti- pyretic and analgesic effects. These effects maybe because of the presence of phytochemicals such as flavonoids, tannins and terpenoids present in the plant extract

The Present study showed that the ethanolic leaves extract *of Luffa acutangula (L) Roxb,* posses peripheral and central analgesic activity in animal model. The *Luffa acutangula (L) Roxb* leaves extract shows anti-pyretic activity in animal model in rats and *Luffa acutangula (L) Roxb* showed anti-inflammatory activity in different animal model. Flavonoids and tannins are the major constituents of *Luffa acutangula (L) Roxb* leaves, which may be responsible for its Analgesic, Anti-pyretic and Anti-inflammatory activity.

Further detailed study on *Luffa acutangula (L) Roxb plant* using different flogestic agents in this area will enable us to understand the mechanism of action underline the above mention activity.

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# REFERENCE

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